

$$10 \mid 627,141$$

The present invention presents: genomic nucleotide sequence of Borna disease virus, nucleotide and amino acid sequences of Borna disease virus proteins, recombinant viral proteins, vectors and cells containing the sequences or encoding the proteins, ligand binding to these proteins such as antibodies, and the diagnostic and therapeutic uses of the foregoing.

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                  DIAGNOSTICS AND THERAPEUTICS FOR  
                  NERVOUS SYSTEM DISEASES**

          This patent application is a continuation-in-part of  
U. S. patent application Serial No. 08/434,831, filed on  
10 May 4, 1995, which is a continuation-in-part of U. S.  
patent application Serial No. 08/369,822, filed on  
January 6, 1995.

          This invention was made with Government support  
under Grant No. NS29425, awarded by the National  
15 Institutes of Health (NINDS). The Government has certain  
rights in this invention.

**FIELD OF THE INVENTION**

          The present invention relates to the field of  
20 virology, immunology, gene therapy, transplantation of  
viral transfected cells, and *in vivo* chemical delivery.

**BACKGROUND OF THE INVENTION**

          Borna disease is an immune-mediated neurologic  
25 syndrome {Narayan, O., et al., *Science* 220:1401-1403  
(1983)} caused by infection with Borna disease virus  
(BDV). BDV is a neurotropic, nonsegmented and negative-  
strand RNA virus that causes a progressive, immune-  
mediated neurologic disease characterized by disturbances  
30 in movement and behavior {Ludwig, H., et al., *Prog. Med.  
Virol*, 35:107-151}. It causes fatal disease in expensive  
domestic animals. Although natural infection was  
originally considered to be restricted to horses and  
sheep in Southeastern Germany, recent studies suggest  
35 that BDV infects horses in North America {Kao, M., et  
al., *Vet. Rec.*, 132:241-4 (1993)}, cats in Sweden {Lundgren,

A.-L., et al., *Zbl. Vet. Med. [B]*, 40:298-303 (1993)}, ostriches in Israel {Malkinson, M., et al., *Vet. Rec.*, 133:304 (1993)} and some human subjects with neuropsychiatric disorders in Europe and North America  
5 {Bode, L., et al., *Arch. Virol. [Suppl]*, 7:159-167 (1993); Bode, L., et al., *Lancet*, ii:689 (1988); Fu, Z. F., et al., *J. Affect. Disorders*, 27:61-68 (1993) and Rott, R., et al., *Science*, 228:755-756 (1985)}.

Experimental infection in rats {Narayan, O., et al.,  
10 *Science*, 220:1401-1403 (1983)} results in a multiphasic syndrome characterized by hyperactivity, stereotyped behaviors, dyskinesias and dystonias.

Though natural infection has not been reported in primates, subhuman primates can be infected  
15 experimentally {Sprankel, H., et al., *Med. Microbiol. Immunol.* 165:1-18 (1978) and Stitz, L., et al., *J. Med. Virol.* 6:333-340 (1980)}. Antibodies to BDV proteins have been found in patients with neuropsychiatric disorders {Rott, R., et al., *Science* 228:755-756 (1985); Fu, Z. F., et al., *J. Affective*  
20 *Disord.* 27:61-68 (1993) and Bode, L., et al., *Arch. Virol. (Suppl.)* 7:159-167 (1993)}.

Because BDV grows only to low titer, it was difficult to purify for analysis. However, the identification of BDV cDNA clones by subtractive  
25 hybridization {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA* 87:4184-4188 (1990) and VandeWoude, S., et al., *Science* 250:1276-1281 (1990)} and, more recently, the advent of a method for isolation of virus particles {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 89:11486-11489 (1992)} led to  
30 partial characterization of BDV as a negative-strand RNA virus which transcribes its RNA in the cell nucleus {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 89:11486-11489 (1992)}.

The diagnosis of BDV infection is based on the  
35 appearance of a clinical syndrome consistent with

disease, and the presence of serum antibodies that detect viral proteins in infected cells by indirect immunofluorescent test (IFT) {Pauli, G., et al., *Zbl. Vet. Med. [B]* 31:552-557 (1984)}, Western blot (WB; immunoblotting) or immunoprecipitation (IP) {Ludwig, H., et al., *Prog. Med. Virol.* 35:107-151 (1988)}. These methods are cumbersome and difficult to use for large surveys of human and livestock populations.

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#### SUMMARY OF THE INVENTION

One aspect of the invention presents the nucleotide and amino acid sequences of Borna disease virus (BDV), their derivatives, the vectors for expressing them, and cells transfected by these vectors.

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Another aspect of the invention presents novel BDV viral proteins gp18 and p57 and their respective recombinant proteins, recp18 and recp57. Also disclosed are their nucleotide and amino acid sequences, vectors encoding them, cells transfected by these vectors, and antibodies directed to these proteins.

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Another aspect of the invention presents assays for detecting ligands which bind BDV proteins or their derivatives. Preferably, these assays are immunoassays for detecting antibodies to BDV protein or its derivatives. The assays are useful for detecting: (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. Preferably, p40, p23 or gp18, and their synthetic versions or fragments are used in these assays. The preferred immunoassays are enzyme-linked immunosorbent assays (ELISAs) based on the use of recombinant viral proteins: recp40, recp23, and/or recp18, and/or the immunoreactive fragments of the foregoing, to detect ligands, such as antibodies, in the patient's biological sample, that are immunoreactive with these proteins. The assay can also be used to monitor the diseases by

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monitoring the titer of such ligands. The titer of the ligands can also be prognosticative of the diseases.

Another aspect of the invention presents alternative methods for detecting the above diseases by detecting the  
5 hybridization of nucleotide sequences in a patient's biological sample with the nucleotide sequences coding for BDV protein or its derivatives.

Another aspect of the invention presents assay kits for the above diagnostic tests.

10 Another aspect of the invention presents vaccines against the above diseases.

Another aspect of the invention presents synthetic peptides, based on truncated BDV protein, useful for immunoassays for detecting antibodies to BDV or for  
15 raising antibodies for the therapeutic uses described in the next paragraph. The method for obtaining these peptides are also presented.

Another aspect of the invention presents methods, using ligands or chemicals such as antibodies, capable of  
20 binding to BDV proteins or their derivatives, for treating: (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. Examples of such antibodies are those specific to gp18 and p57. Also presented are these  
25 therapeutic agents, methods for screening for them, especially those that bind to the immunogenic epitopes of BDV protein. The methods for producing the antibodies are also presented.

Another aspect of the invention presents a BDV-based  
30 viral vector useful for *in vivo* delivery of genes and chemicals to the nervous system. Also disclosed are: the cells transfected by the viral vector and cell lines derived therefrom, the *in vitro* harvesting of the gene product from such cells and cell lines, and the  
35 transplant of such cells into animals.

Other aspects and advantages of the invention will be apparent to those skilled in the art upon

consideration of the following detailed description which provides illustrations of the invention in its presently preferred embodiments.

5                    Brief Description of the Drawings

FIG. 1 presents the genomic organization and transcriptional map of BDV.

FIG. 2 shows the complete genomic sequence of BDV (strain V) in 5' to 3' cDNA with the deduced amino acid  
10                    sequence shown below the cDNA.

FIG. 3 (a) presents the organization of the BDV genome; (b) presents the coding potential of the genome.

FIG. 4 shows alignment of the p180 (also referred to as "pol") open reading frame (ORF) and negative-strand  
15                    RNA virus L-polymerase amino acid sequences with PILEUP computer program (Sequence Analysis Software Package, Genetics Computer, Inc., Madison, Wisconsin). BDV sequence is indicated with double arrowheads.  
Rhabdoviridae: RaV, rabies virus; VSV, vesicular  
20                    stomatitis virus; SYN, sonchus yellow net virus. Paramyxoviridae: MeV, measles virus; SeV, Sendai virus; NDV, Newcastle disease virus; RSV, respiratory syncytial virus. Filoviridae: MaV, Marburg virus.

FIG. 5 presents sequence analysis of BDV genomic  
25                    termini. (a) Similarity of 3'-terminal BDV sequence to leader regions of Rhabdoviridae (RaV), VSV), Paramyxoviridae (MeV, SeV, NDV, RSV), and Filoviridae (MaV); (b) Comparison of complementarity at 3' and 5' termini of BDV genomic RNA with that of four other  
30                    nonsegmented, negative-strand RNA viruses.

FIG. 6 presents the map of BDV subgenomic RNAs relative to the viral antigenome. (a) Northern hybridization analysis of rat brain poly(A)<sup>+</sup> RNA; (b) position of viral transcripts with respect to antigenome  
35                    as determined by Northern hybridization and sequence analysis; (c) alignment of the seven potential termination sites of BDV.

FIG. 7 presents the sequence of ORF gp18.

FIG. 8 shows glycan determination of gp18. Lanes: 0, protein detection by mouse anti-gp18 serum; 1, ConA; 2, wheat germ agglutinin; 3, *D. stramonium* agglutinin; 4, 5 BS-I; 5, BS-II; 6, *G. nivalis* agglutinin; 7, *S. nigra* agglutinin; 8, *M. amrensis* agglutinin; 9, peanut agglutinin. Positions of molecular weight markers are shown in kilodaltons at the right.

FIG. 9 presents treatment of gp18 with buffer alone or endoglycosidase. Lanes: 1, buffer; 2, endoglycosidase F plus N-glycosidase F; 3, endoglycosidase F (N-glycosidase free); 4, endo- $\beta$ -galactosidase. Positions of molecular weight markers are shown in kilodaltons at the right.

FIG. 10 presents *in vitro* transcription, translation, and cotranslational processing of gp18. (A) Lanes: 1, pBDV-23 RNA; 2, pBDV-23 RNA plus microsomal membranes; 3, pBDV-gp18 RNA; 4, pBDV-gp18 RNA plus microsomal membranes; 5, pBDV-gp18 RNA plus microsomal membranes, 20 incubated with endoglycosidases. (B) Lanes: 1, pBDV-gp18 RNA; 2, pBDV-gp18 RNA plus microsomal membranes; 3, pBDV-gp18 RNA plus microsomal membranes, incubated with endoglycosidases.

FIG. 11 presents Western blot analysis of native and 25 recombinant proteins with monospecific antisera to recombinant proteins and sera from infected rats. (A) Lane 1, C6BDV lysate; lane 2, recp40; lane 3, recp23; lane 4, recp18; lane 5, C6 lysate; lane 6, recp40, recp23 and recp18. Lanes 1-4 were treated with serum from 30 infected rat; lanes 5 and 6 were treated with serum from noninfected rat. (B) C6BDV lysates (lanes 1-3) and C6 lysates (lanes 4 and 5) were incubated with: lanes 1 and 4, serum from infected rat; lane 2, anti-p40 rabbit serum; lane 3, anti-p23 rabbit serum; and lane 5, pooled 35 anti-p40 and anti-p23 sera.



FIG. 12 presents ELISA of infected rat serum reacted with recp40. Circles, recp40 and serum from chronically infected rat; squares, recp40 and serum from noninfected rat; triangles, BSA and serum from chronically infected rat.

FIG. 13 presents timecourse for the appearance of antibodies to BDV-proteins. (A) recp40; (B) recp23; and (C) recp18.

FIG. 14 presents timecourse for the appearance of antibodies to BDV proteins in sera from individual rats after intranasal infection. (A) Neutralization activity in sera from BDV-infected rats at three timepoints (5, 10 and 15 weeks post-infection). (B) Plot of mean recp18 ELISA titers (open columns) with neutralization titers (hatched columns) at three time points (5, 10 and 15 weeks post-infection). Sera analyzed were the same as those in panel A. (C) Timecourse for the appearance of antibodies to recp40, recp23, and gp18 by Western blot analysis.

FIG. 15 presents (A) Immunoprecipitation of gp18 with monoclonal antibodies (MAbs). Lanes: 1, serum from infected rat (15 week pi); 2, serum from noninfected rat; 3, MAb 14/29A5; 4, MAb 14/26B9; 5, MAb 14/8E1; 6, MAb 14/13E10; 7, MAb 14/18H7; 8, MAb 24/36F1 (MAb directed against the BDV 23 kDa protein, negative control); 9, no antibody. (B) MAbs were analyzed for binding to native gp18 in Western blot. Lanes: 1, serum from infected rat (15 week p.i., D2); 2, serum from noninfected rat; 3, MAb 14/29A5; 4, MAb 14/26B9; 5, MAb 14/8E1; 6, MAb 14/13E10; 7, MAb 14/18H7; and 8, MAb 24/36F1 (MAb directed against the BDV 23 kDa protein, negative control).

FIG. 16 presents neutralization profile of sera and MAbs. (A) Serum from noninfected rat. (B) serum from infected rat (15 week p.i., D2). (C) MAb 14/13E10. (D) MAb 14/29A5.

FIG. 17 presents precipitation of BDV with sera from infected rats. (A) Lanes: 1, serum from infected rat, 15

week p.i.; 2, serum from infected rat, 5 week p.i.; 3, serum from infected rat, 15 week p.i., no BDV; 4, serum from infected rat, 15 week p.i., genome sense primer used for first strand cDNA synthesis. (B) Precipitation of  
5 BDV by monospecific antisera to recp18 and MABs to gp18. Lanes: 1, monospecific rat antisera to recp18; 2, MAB 14/13E10; 3, MAB 14/29A5. DNA markers (basepairs) are shown at the right.

FIG. 18 presents the cDNA of BDV polymerase. "V" denotes the site of its intron which is located between nucleotide positions 2410 and 3703 in the figure. "I-2", denotes that this is the second intron in the BDV genome.

FIG. 19 presents the partial cDNA genomic sequence for BDV strain HE/80.

15 FIG. 20 graphically presents in A) the immunoreaction of truncated recp23 protein fragments with sera from 7 human schizophrenics (SZ Human), 4 BDV infected horses (BD Horse) and 6 BDV infected rats (BD Rat); and in B) the truncated recp23 fragments.

20 FIG. 21 graphically presents in A) the immunoreaction of truncated unglycosylated recp18 protein fragments with sera from 7 human schizophrenics (SZ Human), 6 BDV infected rats (BD Rat) and 2 mice immunized with native gp18 (Mouse  $\alpha$  gp18); and in B) the truncated  
25 unglycosylated recp18 fragments.

FIG. 22 graphically presents the overlapping 8-mer peptides, derived from p23, lined up diagonally from the amino (left) terminus to the carboxyl (right) terminus. Above the overlapping peptides are blocks indicating the  
30 immunoreactive regions of p23 and presenting the mapped epitopes and their sequences.

FIG. 23 graphically presents the overlapping 8-mer peptides, derived from unglycosylated recp18, lined up diagonally from the amino (left) terminus to the carboxyl  
35 (right) terminus. Above the overlapping peptides are blocks indicating the immunoreactive regions of

unglycosylated gp18 and presenting the mapped epitopes and their sequences.

FIG. 24 graphically presents A) the SPOTs tests; B) the locations of immunoepitopes along the length of unglycosylated gp18 which are immunoreactive with the sera in the SPOTs tests of FIG. 24A. The sequences of the most immunoreactive epitopes are shown. The scale indicates by the darkness of the spots, the degree of immunoreaction. The lightest shade (Scale 1) indicates no detectable immunoreactivity; the darkest shade (Scale 4) indicates highest immunoreactivity.

FIG. 25 presents the predicted amino acid sequence and potential N-glycosylation sites of the BDV G-protein.

#### 15 DETAILED DESCRIPTION OF THE INVENTION

##### BDV Protein, its Amino Acid and Nucleotide Sequences

Table 1 identifies the sequence ID Nos. with their respective nucleotide and amino acid sequences.

20 Table 1  
Nucleotide and Amino Acid Sequences of Borna Disease

##### Virus (BDV)

25	<u>Nucleotide Sequence</u>	<u>Sequence ID No.</u>
	p40	1
	p23	3
	gp 18	5
30	p57	7
	BDV polymerase	9
	BDV genomic cDNA	19
	<u>Amino Acid Sequence</u>	<u>Sequence ID No.</u>
35	p40	2
	p23	4
	gp 18	6
	p57	8
40	BDV polymerase	10

BDV polymerase is also referred to as "pol" or "p180".

The present application discloses the complete BDV  
45 genomic nucleotide sequence, the locations on the genomic

nucleotide sequence which encode the different BDV proteins, the sites of splicing and overlap (see FIGs. 1 and 2). Also disclosed are the novel nucleotide and amino acid sequences of BDV proteins gp18, pol and p57. The following Figures 1, 2, 19, and Table 1 summarize this information.

FIG. 1 shows the genomic organization and transcriptional map of BDV. The BDV genome is shown as a solid line in 3' to 5' direction. Coding regions and their respective reading frames are represented as boxes at the top; the number above each upward vertical line indicates the nucleotide position of the first AUG codon in the respective ORF. Transcription initiation sites and their nucleotide positions on the viral genome (BDV strain V) are represented by arrows pointing downstream. Transcription termination sites and splice sites are indicated by downward vertical lines. Dashed lines indicate that readthrough at termination sites T2 and T3 results in synthesis of longer RNAs terminating at T3 and T4, respectively. The 1.2 kb and 0.8 kb RNA have been shown to represent the mRNAs for p40 and p23, respectively. p23 could also be translated from the 3.5 kb RNA. Transcripts that are likely to represent mRNAs for gp18, p57 and pol are indicated. Note that gp18 can only be translated from RNAs containing intron 1. Splicing of intron 1 preserves the gp18 initiation codon but introduces a stop codon such that only the first 13 amino acids could be translated from the 2.7 (7.0) kb transcripts and the RNA or the 1.4 kb RNA serve as messages for the translation of BDV proteins.

FIG. 2 shows the complete genomic sequence of BDV (strain V) in 5' to 3' cDNA. The deduced amino acid sequences are shown for p40, p23, gp18, p57 and pol. Note: the full amino acid sequence for pol after splicing modification is shown in sequence ID No. 10. The stars (\*) indicate stop codons. Information on transcription and splicing of the genomic sequence is

found in Schneider, P.A. *et al.*, *J. Virol.*, 68:5007-5012 (1994) and Schneemann, A., *et al.*, *J. Virol.*, 68:6514-6522 (1994), both references are hereby incorporated by reference in their entirety.

5        FIG. 19 presents the partial cDNA genomic sequence (also listed as SEQ ID No. 33) of BDV strain HE/80. Position 1 to 2651 of this sequence corresponds to position 1397 through 4054 of the cDNA genomic sequence of BDV strain V. The cDNA sequence of BDV strain HE/80  
10        disclosed herein encodes part of the p23 and BDV polymerase proteins, and the complete gp18 and p57 proteins.

      The term "nucleotide sequence" as used herein, unless otherwise modified, includes both ribonucleic acid  
15        (RNA) and deoxyribonucleic acid (DNA).

      The sequences in Table 1 include both native and synthetic sequences. Unless otherwise modified, the term "protein" as used herein encompasses both native and synthetic polypeptide and peptide. Synthetic protein  
20        includes recombinant and chemically synthesized protein. Unless otherwise indicated, "gp18", "p57", and "pol" proteins include both their native and synthetic versions. "recp18", "recp57" and "recpol" are recombinant proteins of "gp18", "p57", and "pol"  
25        proteins, respectively. The terms "p57" and "recp57" herein include both the predicted protein of about 57 kDa, and the glycoprotein of about 94 kDa (G-protein), further described below.

      Some of the nucleotide sequences disclosed are in  
30        the form of DNA. For example, SEQ ID No. 19 presents the BDV viral genomic sequence as cDNA of BDV viral genomic RNA. One skilled in the art would realize that the BDV viral genomic RNA is complementary to its cDNA that is shown in Figure 2. The term "BDV genomic nucleotide  
35        sequence" thus includes both the full cDNA and RNA sequences of the BDV genome. Further, as used in this application and claims, the SEQ ID Nos. and disclosed

sequences include: (1) the DNA sequences as disclosed, (2) the complementary nucleotide sequences (which may be RNA or DNA) to the disclosed sequences, (3) the corresponding RNA sequences to the listed DNA sequences wherein the Thymidine ("T") in the disclosed DNA sequences is replaced with Uracil ("U"), (4) nucleotide sequences wherein other nucleotides known in the art such as nucleotide analogs, replace those in the foregoing sequences, for example, 5-methyl-cytosine replacing cytosine, and (5) nucleotide sequences that are within a variance (with regard to the respective SEQ ID Nos. or disclosed nucleotide sequences) of at least about: 10%, preferably 28%, more preferably 30%, and most preferably 35%. For example, Kishi, M., *et al.*, "Sequence Variability of Borna Disease Virus Open Reading Frame II Found in Human Peripheral Blood Mononuclear Cells", *J. Virol.*, 70(1):635-640 (Jan. 1996), cloned, sequenced, and analyzed cDNA of BDV ORF-II which encodes p24, from the peripheral blood mononuclear cells of three psychiatric patients. Fifteen clones were studied. Intrapatient divergences of the BDV ORF-II nucleotide sequence were 4.2% to 7.3%, 4.8% to 7.3%, and 2.8% to 7.1% of the three patients, leading to differences of 7.7% to 14.5%, 10.3% to 17.1%, and 6.0% to 16.2%, respectively, in the deduced amino acid sequence for BDV p24. Interpatient divergencies among the 15 clones were 5.9% to 12.7% at the nucleotide level and 12.8% to 28.2% at the amino acids level. The nucleotide sequences of the 15 human BDV ORF-II cDNA clones differed from those of horse strains V and He/80-1 by 4.2% to 9.3%. This reference is hereby incorporated by reference in its entirety. The above discussion would analogously apply to RNA sequences disclosed in this application.

Since nucleotide codons are redundant, also within the scope of this invention are equivalent nucleotide sequences which include: nucleotide sequences which code for the same proteins or equivalent proteins. Thus,

nucleotide sequences which encode substantially the same or functionally equivalent amino acid sequence may be used in the practice of the invention.

The terms "BDV genomic nucleotide sequence", "p18", "recp18", "pol", "recpol", "p57", "recp57", as used in relation to nucleotide sequences are defined above, together with: (1) nucleotide sequences that are within a variance (with regard to the respective nucleotide sequences in Table 1) of at least about: 10%, preferably 28%, more preferably 30%, and most preferably 35% (see also the discussion of Kishi, M., *et al. J. Virol.*, 70(1), above); (2) nucleotide sequences that are capable of hybridizing to the coding sequences of the respective nucleotide sequences, under stringent hybridization conditions, (3) nucleotide sequences coding for gp18, recp18, p57, recp57, pol, and recpol proteins, and amino acid sequences of SEQ ID Nos. 6, 8, and 10 respectively; and (4) fragments of SEQ ID Nos. 6; 8; 10; nucleotide number 1 through 53 and nucleotide number 1880 through 8910 of SEQ ID NO 19 and their fragments; or other nucleotide sequences which, for example, encode proteins having substantially the same biological characteristics/activities of gp18, recp18, p57, recp57, pol, recpol proteins, respectively. Preferably, the determinative biological characteristic/activity is the retention of at least one immunoepitope. Preferably, when used in an immunoassay for BDV, these proteins are immunoreactive with antibodies directed to BDV but not detectably immunoreactive with non-BDV specific antibodies found in a biological sample such as a serum sample. Alternatively, the nucleotide sequences can be nucleotide probes of at least 10 nucleotides in length. Preferably, when used in a hybridization assay for BDV, these probes do not detectably hybridize to the nucleotide sequences of non-BDV organisms which are found in a biological sample such as a serum sample. Alternatively, the nucleotide sequences hybridize to at

least 10 consecutive nucleotides in the coding sequences of the above listed nucleotide sequences. The nucleotide sequences include a nucleotide sequence which encodes a protein containing at least 8; more preferably, 5 to 6; and most preferably, 4 amino acids. Preferably, the protein is specific to BDV or retain one or more biological functions of BDV. Examples of such biological functions are: BDV's ability to bind a particular cellular receptor, BDV's ability to target its host cells (e.g. cells and tissues of the nervous system, bone marrow, peripheral blood, mononuclear cells or brain), and BDV's effects on the functions of cells infected by it. The discussion herein similarly applies to p23, recp23, p80, recp80 nucleotide sequences, and the cDNA nucleotide sequence of FIG. 19, e.g. in reference to their respective SEQ ID NOs and FIG. 19.

The terms "gp18", "recp18", "p57", "recp57", "pol", and "recpol", as used in relation to proteins are, respectively, as defined above together with: (1) protein variants containing amino acid sequences that are within a variance (with regard to the amino acid sequences of SEQ ID Nos. 6, 8, and 10, respectively) of at least about: 5%, preferably 28%, more preferably 30%, and most preferably 35% (see also the discussion of Kishi, M., *et al. J. Virol.*, 70(1), above); (2) the functional equivalents of these proteins and their variants, respectively; and (3) the derivatives, including fragments, of gp18, recp18, p57, recp57, pol, recpol, proteins and their variants, respectively. Preferably, when used in an immunoassay for BDV, these proteins are immunoreactive with antibodies directed to BDV but not detectably immunoreactive with non-BDV specific antibodies found in a biological sample such as a serum sample. Alternatively, these proteins each contains at least 8; more preferably, 5 to 6; and most preferably, 4 amino acids. Preferably, the latter proteins are specific to BDV or retain one or more biological



functions of BDV. Examples of such biological functions are: BDV's ability to bind a particular cellular receptor, BDV's ability to target its host cells (*e.g.* cells and tissues of the nervous system, bone marrow, peripheral blood, mononuclear cells or brain), and BDV's effects on the functions of cells infected by it. The discussion herein similarly applies to p23, recp23, p80, and recp80 proteins, *e.g.* in reference to their respective SEQ ID NOs.

10        Within the definition of "BDV" are BDV isotypes, strains, and BDV related viruses. The term "BDV proteins and their derivatives", includes BDV proteins, fragments of BDV proteins, proteins containing immunoepitopes of BDV, variants and functional equivalents of the foregoing. gp18 and p57 are examples of BDV proteins. 15        Preferably, the immunoepitope is specific to BDV.

      The variants can result from, *e.g.* substitution, insertion, or deletion of the amino acid sequences shown in Table 1. The derivatives of the proteins and their 20        variants, include fragments of these proteins and their immunogenic epitopes. Preferably, each of the fragments contains at least one immunogenic epitope of BDV. More preferably, the fragment is capable of being bound by polyclonal antibodies directed to BDV. In the case of 25        antibodies which recognize linear epitopes, they generally bind to epitopes defined by about 3 to 10 amino acids. Preferably, too, each variant retains at least one immunoepitope of BDV. Preferably the immunoepitope is specific to BDV.

30        Two amino acid sequences are functionally equivalent if they have substantially the same biological activities. The proteins may be fused to other proteins, for example, signal sequence fusions may be employed in order to more expeditiously direct the secretion of the 35        BDV protein. The heterologous signal replaces the native BDV signal, and when the resulting fusion is recognized, *i.e.* processed and cleaved by the host cell, the BDV

protein is secreted. Signals are selected based on the intended host cell, and may include bacterial, yeast, insect, mammalian, and viral sequences. For example, the native BDV signal or the herpes gD glycoprotein signal is  
5 suitable for use in mammalian expression systems.

Substitutional variants of the proteins disclosed herein are those in which at least one residue in the disclosed sequences has been removed and a different residue inserted in its place. Preferably, the amino  
10 acid change is conservative. For example, such substitutions generally are made in accordance with the following Table 2.

TABLE 2

	Original Residue	Exemplary Substitutions
	Ala	ser
	Arg	lys
5	Asn	gln; his
	Asp	glu
	Cys	ser; ala
	Gln	asn
	Glu	asp
10	Gly	pro
	His	asn; gln
	Ile	leu; val
	Leu	ile; val
	Lys	arg; gln; glu
15	Met	leu; ile
	Phe	met; leu; tyr
	Ser	thr
	Thr	ser
	Trp	tyr
20	Tyr	trp; phe
	Val	ile; leu

Novel amino acid sequences, as well as isosteric analogs (amino acid or otherwise), are included within  
 25 the scope of this invention.

A variant typically is made by site specific mutagenesis of the encoding nucleic acid, expression of the variant nucleic acid in recombinant cell culture and, optionally, purification from the cell culture for  
 30 example by immunoaffinity adsorption on a column to which are bound polyclonal antibodies directed against the original protein from which the variant is derived.

Another class of variants are deletional variants. Deletions are characterized by the removal of one or more  
 35 amino acid residues from the original protein sequence. Typically, deletions are used to affect the original protein's biological activities. However, deletions which preserve the biological activity or immune cross-reactivity of the original protein are preferred.

40 Deletions of cysteine or other labile residues also may be desirable, for example in increasing the oxidative stability of the original protein. Deletion or substitutions of potential proteolysis sites, e.g. Arg Arg,

is accomplished by deleting one of the basic residues or substituting one by glutaminy or histidyl residues.

It will be understood that some variants may exhibit reduced or no biological activity. These variants  
5 nonetheless are useful as standards in immunoassays for BDV protein so long as they retain at least one immunogenic epitope of BDV protein.

It is presently believed that the three-dimensional structure of the proteins of the present invention is  
10 important to their functioning as described herein. Therefore, all related structural analogs which mimic the active structure of those formed by the compositions or proteins claimed herein are specifically included within the scope of the present invention.

15 Modified proteins are also within the contemplation of this patent application. These modifications may be deliberate, e.g., modifications obtained through site-directed mutagenesis, or may be accidental, e.g., as those obtained through mutation of the hosts.

20 Further, as is the case for all proteins, the precise chemical structure depends on a number of factors. As ionizable amino and carboxyl groups are present in the molecule, a particular protein may be obtained as an acidic or basic salt, or in neutral form.  
25 All such preparations which retain their activity when placed in suitable environmental conditions are included in the definition. Additionally, the primary amino acid sequence may be augmented by derivatization using sugar moieties (glycosylation) or by other supplementary  
30 molecules such as lipids, phosphate, acetyl groups and the like, more commonly by conjugation with saccharides. The primary amino acid structure may also aggregate to form complexes, most frequently dimers. Certain aspects of such augmentation are accomplished through post-  
35 translational processing systems of the producing host; other such modifications may be introduced *in vitro*. In any event, such modifications are included in the definition

so long as the activity of the protein is not destroyed. It is expected that such modifications may quantitatively or qualitatively affect the activity, either by enhancing or diminishing the activity of the protein in various  
5 assays.

Individual amino acid residues in the chain may also be modified by oxidation, reduction, or other derivatization, and the protein may be cleaved to obtain fragments which retain activity. Such alterations which  
10 do not destroy activity do not remove the protein sequence from the definition. The following discusses some of the modifications in further detail by way of example.

Thus, glycosylation variants are included within the  
15 scope of BDV. They include variants completely lacking in glycosylation (unglycosylated) and variants having at least one less glycosylated site than the native form (deglycosylated) as well as variants in which the glycosylation has been changed. Included are  
20 deglycosylated and unglycosylated amino acid sequence variants, deglycosylated and unglycosylated BDV and gp18 having the native, unmodified amino acid sequence of BDV and gp18, and other glycosylation variants, *e.g.* of p57. For example, substitutional or deletional mutagenesis is  
25 employed to eliminate the N- or O-linked glycosylation sites of BDV or gp18, *e.g.*, an asparagine residue is deleted or substituted for by another basic residue such as lysine or histidine. Alternatively, flanking residues making up the glycosylation site are substituted or  
30 deleted, even though the asparagine residues remain unchanged, in order to prevent glycosylation by eliminating the glycosylation recognition site.

Unglycosylated protein which has the amino acid sequence of the native protein is preferably produced in  
35 recombinant prokaryotic cell culture because prokaryotes are incapable of introducing glycosylation into polypeptides.

Glycosylation variants are produced by selecting appropriate host cells or by *in vitro* methods. Yeast, for example, introduce glycosylation which varies significantly from that of mammalian systems. Similarly, mammalian cells having a different species (e.g. hamster, murine, insect, porcine, bovine or ovine) or tissue origin (e.g. lung, liver, lymphoid, mesenchymal or epidermal) than the source of the BDV antigen are routinely screened for the ability to introduce variant glycosylation as characterized for example by elevated levels of mannose or variant ratios of mannose, fucose, sialic acid, and other sugars typically found in mammalian glycoproteins. *In vitro* processing of the proteins of the present invention typically is accomplished by enzymatic hydrolysis, e.g. endoglycosidase digestion.

Derivatization with bifunctional agents is useful for preparing intermolecular aggregates of BDV proteins and their derivatives with polypeptides as well as for cross-linking the protein and derivatives to a water insoluble support matrix or surface for use in the assay or affinity purification of its ligands. In addition, a study of intrachain cross-links will provide direct information on conformational structure. Commonly used cross-linking agents include sulfhydryl reagents, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example esters with 4-azidosalicylic acid, homobifunctional imidoesters including disuccinimidyl esters such as 3,3'-dithiobis (succinimidyl-propionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane.

Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues.

Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other post-translational modifications include  
5 hydroxylation of proline and lysine, phosphorylation of  
hydroxyl groups of seryl or threonyl residues,  
methylation of the  $\alpha$ -amino groups of lysine, arginine,  
and histidine side chains {T.E. Creighton, *Proteins: Structure  
and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp  
10 79-86 (1983)}, acetylation of the N-terminal amine and,  
in some instances, amidation of the C-terminal carboxyl.

The claimed proteins are preferably produced using recombinant technologies. The nucleotide, e.g., DNA or RNA, sequences which encode the desired polypeptides are  
15 amplified by use of e.g. the polymerase chain reaction in the case of DNA (hereinafter also referred to as "PCR"), and reverse transcriptase-polymerase chain reaction (RT-PCR) in the case of RNA. Oligonucleotide sequences to be used as primers which can specifically bind to the ends of the  
20 regions of interest are synthesized. After the desired region of the gene has been amplified the desired sequence is incorporated into an expression vector which is transformed into a host cell. The nucleotide sequence is then expressed by the host cell to give the desired  
25 polypeptide which is harvested from the host cell. Plant, bacterial, yeast, insect, viral and mammalian expression systems may be used. Vectors which may be used in these expression systems may contain fragments of plant, bacterial, yeast, insect, viral, and/or mammalian  
30 origins.

Given the teachings contained herein, one skilled in the art can create the sequences disclosed herein, either by hand or with an automated apparatus. As examples of the current state of the art relating to polynucleotide  
35 synthesis, one is directed to Maniatis *et al.*, *Molecular Cloning--A Laboratory Manual*, Cold Spring Harbor Laboratory (1984),

and Horvath *et al.*, *An Automated DNA Synthesizer Employing Deoxynucleoside 3'-Phosphoramidites*, *Methods in Enzymology* 154: 313-326, 1987.

Identification of Nucleotide Sequences, Cloning, and  
5 Expression of the Disclosed Protein

Alternatively, to obtain RNA encoding the proteins disclosed herein, one needs only to conduct hybridization screening with labelled BDV nucleotide sequence (usually, greater than about 20, and ordinarily about 50bp) in  
10 order to detect clones which contain homologous sequences in the cDNA libraries derived from cells or tissues of a particular animal, followed by analyzing the clones by restriction enzyme analysis and nucleic acid sequencing to identify full-length clones. The cell lines, cells  
15 and tissues are preferably from the nervous system, bone marrow, peripheral blood, mononuclear cells or brain of BDV infected animals. Examples of cells from the nervous system are: neurons, oligodendrocytes and astrocytes. The primers shown in Examples 1 to 4 and/or the methods  
20 shown therein may also be used.

If full length clones are not present in the library, then appropriate fragments are recovered from the various clones and ligated at restriction sites common to the fragments to assemble a full-length clone.

25 The techniques shown in this section are also useful for identifying and sequencing various isotypes and strains of BDV and BDV related viruses. The present invention discloses the nucleotide sequences of two strains of BDV; different strains of BDV may exist or  
30 arise due to mutation as in the case of human immunodeficiency virus (HIV) which constantly mutates and of which different strains are constantly being discovered. Thus, within the definition of BDV are other BDV isotypes and strains or viruses related to  
35 BDV ("BDV related viruses"). For example, the next section of the application describes diagnostic assays for BDV or related pathogenesis. The related



pathogenesis include: (1) diseases caused by BDV; (2) opportunistic or attendant diseases arising from BDV infection; and (3) diseases caused by BDV related viruses. The BDV related viruses would be nonsegmented, negative-stranded, neurotropic, post transcriptionally modified (spliced) viruses which share some homology with BDV nucleotide or amino acid sequences. Patients infected by the BDV related viruses would manifest clinical symptoms similar to BDV infected patients, or to that of neurologic or neuropsychiatric diseases.

Thus, DNA or RNA encoding various BDV isotypes and strains, and BDV related viruses, can be similarly obtained by probing libraries from cells and tissues, especially cells of the nervous system, of animals exhibiting clinical symptoms of BDV infection, neurologic or neuropsychiatric disease; or animals that have been purposely infected with BDV strains, isotypes or BDV related viruses, such as shown in Example 2. Once the DNA or RNA sequence of these strains, isotypes, or related viruses are known, primers based on the sequence may be used. The methods shown in Examples 1 and 2, and the primers shown therein may also be used to obtain the genomic nucleotide sequences.

In general, prokaryotes are used for cloning of DNA sequences in constructing the vectors useful in the invention. For example, *E. coli* K12 strain 294 (ATCC No. 31446) is particularly useful. Other microbial strains which may be used include *E. coli* B and *E. coli* X1776 (ATCC No. 31537). These examples are illustrative rather than limiting. Alternatively, *in vitro* methods of cloning, e.g. polymerase chain reaction, are suitable.

The proteins of this invention may be expressed directly in recombinant cell culture as an N-terminal methionyl analogue, or as a fusion with a polypeptide heterologous to the hybrid/portion, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the hybrid/portion. For

example, in constructing a prokaryotic secretory expression vector for portion/fragment of BDV protein, the native BDV signal is employed with hosts that recognize that signal. When the secretory leader is "recognized" by the host, the host signal peptidase is capable of cleaving a fusion of the leader polypeptide fused at its C-terminus to the desired mature BDV protein. For host prokaryotes that do not process the BDV signal, the signal is substituted by a prokaryotic signal selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp or heat stable enterotoxin II leaders. For yeast secretion the BDV signal may be substituted by the yeast invertase, alpha factor or acid phosphatase leaders. In mammalian cell expression, the native signal is satisfactory for mammalian BDV, although other mammalian secretory protein signals are suitable, as are viral secretory leaders, for example the herpes simplex gD signal.

The proteins of the present invention may be expressed in any host cell, but preferably are synthesized in mammalian hosts. However, host cells from prokaryotes, fungi, yeast, insects and the like are also used for expression. Exemplary prokaryotes are the strains suitable for cloning as well as *E. coli* W3110 (F- $\lambda$ -A-prototrophic, ATTC No. 27325), other enterobacteriaceae such as *Serratia marcescans*, bacilli and various pseudomonads.

Expression hosts typically are transformed with DNA encoding the proteins of the present invention which has been ligated into an expression vector. Such vectors ordinarily carry a replication origin (although this is not necessary where chromosomal integration will occur). Expression vectors also include marker sequences which are capable of providing phenotypic selection in transformed cells, as will be discussed further below. For example, *E. coli* is typically transformed using pBR322,

a plasmid derived from an *E. coli* species {Bolivar, *et al.*, *Gene* 2:95 (1977)}. pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells, whether for purposes  
5 of cloning or expression. Expression vectors also optimally will contain sequences which are useful for the control of transcription and translation, *e.g.*, promoters and Shine-Dalgarno sequences (for prokaryotes) or promoters and enhancers (for mammalian cells). The  
10 promoters may be, but need not be, inducible; even powerful constitutive promoters such as the CMV promoter for mammalian hosts may produce BDV proteins without host cell toxicity. While it is conceivable that expression vectors need not contain any expression control,  
15 replicative sequences or selection genes, their absence may hamper the identification of transformants and the achievement of high level peptide expression.

Promoters suitable for use with prokaryotic hosts illustratively include the  $\beta$ -lactamase and lactose  
20 promoter systems {Chang *et al.*, *Nature* 275:615 (1978); and Goeddel *et al.*, *Nature* 281:544 (1979)}, alkaline phosphatase, the tryptophan (*trp*) promoter system (Goeddel, *Nucleic Acids Res.* 8:4057 (1980) and EPO Appln. Publ. No. 36,776) and hybrid promoters such as the *tac* promoter {H. de Boer *et al.*, *Proc. Natl. Acad. Sci. USA* 80:21-25 (1983)}. However, other  
25 functional bacterial promoters are suitable. Their nucleotide sequences are generally known, thereby enabling a skilled worker operably to ligate them to DNA encoding the proteins of the present invention  
30 {Siebenlist *et al.*, *Cell* 20:269 (1980)} using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the proteins of the present invention

35 In addition to prokaryotes, eukaryotic microbes such as yeast or filamentous fungi are satisfactory.

*Saccharomyces cerevisiae* is the most commonly used eukaryotic microorganism, although a number of other strains are commonly available. The plasmid YRp7 is a satisfactory expression vector in yeast {Stinchcomb, *et al.*, *Nature* 282:39  
5 (1979); Kingsman *et al.*, *Gene* 7:141 (1979); Tschemper *et al.*, *Gene* 10:157 (1980)}. This plasmid already contains the *trp1* gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC no. 44076 or PEP4-1 {Jones,  
10 *Genetics* 85:12 (1977)}. The presence of the *trp1* lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Alternatively, viral expression vectors such as  
15 retroviral vectors, baculoviral vectors and Semliki Forest viral vectors are used. The expression hosts of these vectors are known in the art.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase  
20 {Hitzeman *et al.*, *J. Biol. Chem.* 255:2073 (1980)} or other glycolytic enzymes {Hess *et al.*, *J. Adv. Enzyme Reg.* 7:149 (1968); and Holland, *Biochemistry* 17:4900 (1978)}, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-  
25 phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucos isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription  
30 controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and  
35 galactose utilization. Suitable vectors and promoters

for use in yeast expression are further described in R. Hitzeman *et al.*, European Patent Publication No. 73,657A.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence which may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences may be inserted into mammalian expression vectors.

Suitable promoters for controlling transcription from vectors in mammalian host cells are readily obtained from various sources, for example, the genomes of viruses such as polyoma virus, SV40, adenovirus, MMV (steroid inducible), retroviruses (e.g. the LTR of BDV), hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. the beta actin promoter. The early and late promoters of SV40 are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication. {Fiers *et al.*, *Nature* 273:113 (1978)}. The immediate early promoter of the human cytomegalovirus is conventionally obtained as a HindIII E restriction fragment. {Greenaway, P.J. *et al.*, *Gene* 18:355-360 (1982)}.

Transcription of a DNA encoding the proteins of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10-300bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' {Laimins *et al.*, *Proc. Natl. Acad. Sci.*, 78:993 (1981)} and 3' {Lusky, M.L., *et al.*, *Mol. Cell Bio.* 3:1108 (1983)} to the transcription unit,

within an intron {Banerji, J.L. *et al.*, *Cell* 33:729 (1983)} as well as within the coding sequence itself {Osborne, T.F., *et al.*, *Mol. Cell Bio.* 4:1293 (1984)}. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription which may affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA. The 3' untranslated regions also include transcription termination sites.

Expression vectors may contain a selection gene, also termed a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase (TK) or neomycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell is able to survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are CHO DHFR- cells and mouse LTK cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the

media is to introduce an intact DHFR or TK gene into calls lacking the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable  
5 of survival in non-supplemented media.

The second category of selective regimes is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest  
10 growth of a host cell. Those cells which are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin {Southern *et al.*, *J. Molec. Appl. Genet.*  
15 1:327 (1982)}, mycophenolic acid {Mulligan *et al.*, *Science* 209:1422 (1980)} or hygromycin {Sugden *et al.*, *Mol. Cell. Biol.* 5:410-413 (1985)}. The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin  
20 (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively.

"Amplification" refers to the increase or replication of an isolated region within a cell's chromosomal DNA. Amplification is achieved using a  
25 selection agent, e.g. methotrexate (MTX) which inactivates DHFR. Amplification or the making of successive copies of the DHFR gene results in greater amounts of DHFR being produced in the face of greater amounts of MTX. Amplification pressure is applied notwithstanding the  
30 presence of endogenous DHFR, by adding ever greater amounts of MTX to the media. Amplification of a desired gene can be achieved by cotransfecting a mammalian host cell with a plasmid having a DNA encoding a desired protein and the DHFR or amplification gene permitting  
35 cointegration. One ensures that the cell requires more DHFR, which requirement is met by replication of the selection gene, by selecting only for cells that can grow

in the presence of ever-greater MTX concentration. So long as the gene encoding a desired heterologous protein has cointegrated with the selection gene replication of this gene gives rise to replication of the gene encoding the desired protein. The result is that increased copies of the gene, i.e. an amplified gene, encoding the desired heterologous protein express more of the desired protein.

Suitable eukaryotic host cells for expressing the proteins include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, {Graham, F.L. et al., *J. Gen Virol.* 36:59 (1977)}; baby hamster kidney cells (BHK, ATCC CCL 10); chinese hamster ovary-cells-DHFR {CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci., (USA)* 77:4216, (1980)}; mouse sertoli cells {TM4, Mather, J.P., *Biol. Reprod.* 23:243-251 (1980)}; monkey kidney cells (CV1 ATCC CCL 70); african green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells {Mather, J.P., et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982)}; and C<sub>6</sub> glial cell (ATCC CCL 107).

Construction of suitable vectors containing the desired coding and control sequences employ standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to form the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction and/or sequenced by the method of Messing et al., *Nucleic Acids*



Res. 9:309 (1981) or by the method of Sanger *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 74:5463 (1977).

Host cells are transformed with the expression vectors of this invention and cultured in conventional  
5 nutrient media modified as appropriate for inducing promoters, selecting transformants or amplifying the genes encoding the desired sequences. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for  
10 expression, and will be apparent to the ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in *in vitro* culture as well as cells which are within a host animal.

15

**Diagnostic, Prognostic, and Monitoring Uses of BDV proteins and their derivatives**

Another aspect of the present invention presents assays for detecting ligands, *e.g.*, in the biological  
20 samples of a test organism, which bind BDV protein(s) or derivatives thereof. These assays are useful as diagnostic tests for: (1) infection by BDV or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection.

25 The preferred assays are immunoassays which detect antibodies to BDV proteins or its derivatives that are antigenic (herein referred to as "BDV antigen"). The test organism can be human or other animals, such as cats, fowls, ostriches, and horses. The biological  
30 samples may be biological fluids such as whole blood, serum, plasma, cerebral spinal fluid, or synovial fluid. Preferably, BDV proteins or its derivatives are used to detect the ligand by binding to it. Preferably, the ligand is an antibody directed to the polypeptides, and  
35 BDV antigens are used to detect the antibody. For example, the assay can be used to detect antibodies against BDV in biological fluids.

Alternatively, antibodies to BDV protein(s) or their derivatives can be used to screen for BDV proteins, e.g., in the biological samples of a test organism. Similarly, the alternative detection of antibodies or antigen  
5 applies to each of the assay formats described below.

Thus, an example of the assay is an enzyme immunoassay. In an example of a direct assay, these polypeptides serve as antigens and are attached to a solid phase and then incubated with patient sera. Human  
10 serum or plasma is preferably diluted in a sample diluent before incubation. If antibodies to BDV are present in the sample they will form an antigen-antibody complex with the polypeptides and become affixed to the solid phase.

15 After the antigen-antibody complex has formed, unbound materials and reagents are removed by washing the solid phase and the antigen-antibody complex is reacted with a solution containing labelled antibodies directed against the first type of antibody. For example, the  
20 labelled antibody can be horseradish peroxidase-labeled goat antibody. This peroxidase labelled antibody then binds to the antigen-antibody complex already affixed to the solid phase. In a final reaction the horseradish peroxidase is contacted with o-phenylenediamine and  
25 hydrogen peroxide which results in a yellow-orange color. The intensity of the color is proportional to the amount of antibody which initially binds to the polypeptide affixed to the solid phase.

Another assay format provides for an antibody-  
30 capture assay in which anti-immunoglobulin antibody on the solid phase captures the patient's antibody, which is then reacted with the BDV antigen. The application of this format is similar to the serological assay of Lyme disease taught in Berardi et al., *J. Infect. Dis.* 158:754-760  
35 (1988). If antibody to BDV is present, it captures the BDV antigen, and the bound BDV antigen is detected by means of labelled polyclonal or monoclonal antibodies

directed against the BDV antigen. The antibody-capture assay is particularly useful for and can increase the sensitivity of detection of IgM and IgG to BDV antigens. In an example of this assay, the fluid sample is first  
5 contacted with a solid support containing a bound antibody capable of binding the mu-chain of IgM or the gamma-chain of IgG antibodies. Specific antibody is detected by reacting this with the BDV antigens followed by non-human antibody to the BDV antigens. The non-human  
10 antibody is generally labelled for detection. It is believed that this antibody-capture immunoassay format will have increased sensitivity, especially for IgM. Alternatively, one can forego the non-human antibody and instead label the BDV antigens for direct detection.

15 Another assay format provides for an immunodot assay for identifying the presence of an antibody that is immunologically reactive with specific BDV antigens by contacting a sample with the BDV antigens bound to a solid support under conditions suitable for complexing  
20 the antibody with the BDV antigens and detecting the antibody-antigen complex by reacting the complex.

Suitable methods and reagents for detecting an antibody-antigen complex in an assay of the present invention are commercially available or known in the  
25 relevant art. For example, the detector antibodies or polypeptides may be labelled with enzymatic, radioisotopic, fluorescent, luminescent, or chemiluminescent label. These labels may be used in hapten-labelled antihapten detection systems according to  
30 known procedures, for example, a biotin-labelled antibiotin system may be used to detect an antibody-antigen complex.

In all of the assays, the sample is preferably diluted before contacting the BDV antigen absorbed on a  
35 solid support. Solid support materials may include cellulose materials, such as paper and nitrocellulose; natural and synthetic polymeric materials, such as

polyacrylamide, polystyrene, and cotton; porous gels such as silica gel, agarose, dextran and gelatin; and inorganic materials such as deactivated alumina, magnesium sulfate and glass. Suitable solid support materials may be used in assays in a variety of well known physical configurations, including microtiter wells, test tubes, beads, strips, membranes, and microparticles. A preferred solid support for a non-immunodot assay is a polystyrene microwell, polystyrene beads, or polystyrene microparticles. A preferred solid support for an immunodot assay is nitrocellulose or nylon membrane.

In particular, the invention presents an ELISA which is a rapid, sensitive, and inexpensive diagnostic test. The preferred ELISAs are based on recombinant BDV proteins recp40, recp23, and recp18. These assays are more sensitive and rapid than prior art methods employed for serologic diagnosis of infection, such as Western blot, indirect immunofluorescent test or immunoprecipitation.

Examples of the neurologic and neuropsychiatric diseases that can be diagnosed include diseases of the nervous system such as schizophrenia, depressive disorders (e.g., bipolar depression), multiple sclerosis and AIDS-related encephalopathy. The finding is based on applicants' analysis of the art. Although the virus has not been recovered from human subjects, antibodies reactive with BDV proteins have been found in patients with bipolar depression, schizophrenia, or AIDS-related encephalopathy {Bode, L., et al., *Arch. Virol. Suppl.*, 7:159-167 (1993); Bode, L., et al., *Lancet*, ii:689 (1988) and Rott, R., et al., *Science* 228:755-756 (1985)}. BDV has a unique tropism for specific brain regions. Viral nucleic acids and disease-specific proteins have been found in highest concentrations in the hippocampus and limbic circuits, prefrontal and cingulate cortices, and brainstem nuclei {Carbone, K., et al., *J. Neuropathol. Exp.*

*Neurol.*, 50:205-214 (1991); Ludwig, H., et al., *Prog. Med. Virol.* 35:107-151 (1988) and Solbrig, M. V., et al., abstr. 10, Abstr. 1992 Am. Acad. Neurol. Annu. Meet., (1992)}. Three BDV proteins, p40, p23 and gp18 (disclosed in  
5 Example 2 below) have been identified in infected cells and tissues {Ludwig, H., et al., *Prog. Med. Virol.* 35:107-151 (1988) and Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)}. cDNAs for p40 {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990); McClure, M. A., et  
10 al., *J. Virol.*, 66:6572-6577 (1992) and Pyper, J. M., et al., *Virology*, 195:229-238 (1993)} and p23 {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990); Thierer, J., et al., *J. Gen. Virol.*, 73:413-416 (1992) and Vandewoude, S., et al., *Science*, 250:1276-1281 (1990)} have been  
15 isolated, and complementary sequences to open reading frames (ORFs) for these proteins have been mapped to the viral genome {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 91:4362-4366 (1994) which is incorporated into Example 1 of this application; and Cubitt, B., et al., *J. Virol.*,  
20 68:1382-1996 (1994)}.

The assay can also be used to monitor the diseases by monitoring the titer of such ligands. The titer of the ligands, and the specific viral proteins that it is immunoreactive with, can also be prognosticative of the  
25 diseases.

Thus, an application of this invention may involve contacting the test subject's biological sample, such as serum, with a panel consisting of different immunogenic fragments of BDV protein or its derivatives. These  
30 proteins may be synthetic or native proteins, though recombinant proteins are preferred. Such a panel may consist of, for example, recp23, recp40, recp57, recpol and recp18. If the serum is immunoreactive with at least one of the fragments, it indicates that the test subject  
35 may either be suffering from (1) BDV or related

pathogenesis; or (2) neurologic and neuropsychiatric disease not due to BDV infection. Further, given a fixed amount of sample tested, the amount (i.e. percentage) of ligands immunoreactive with the BDV proteins may also be indicative of the severity of the disease and thus its prognosis. Generally, the higher the percentage of ligands that are immunoreactive, the more severe the disease and the poorer the prognosis. Thus, the assay may also be used to monitor the progress of the disease. In particular, if the test subject is undergoing treatment for the disease, the assay may be used to monitor the efficacy of the drug and treatment regimen. Such monitoring may involve assaying for the ligand titer and/or the specific BDV immunogenic epitopes which the ligand binds to.

#### Hybridization Diagnostic Assays

Oligonucleotides ("probes") that are unique, or relatively unique to BDV in a test sample, are useful for diagnosing BDV infections. Nucleotide hybridization assay may be used, whereby nucleic acids from a patient's biological sample are contacted to the primers or BDV restriction fragments under hybridization condition, and the hybridization products are detected. This method could be used to detect viral genomic RNA or mRNA. Conventional Western or Northern Blot analysis, RT-PCR or PCR and ligase chain reaction (LCR) may be used as the basis of the assay, these techniques are known to those skilled in the art. PCR and LCR techniques are widely available in the art. For example, the basic PCR techniques are described in United States Patent Nos. 4,683,202; 4,683,195; 4,800,159; and 4,965,188. The basic LCR techniques are described in EPA-320,308; EPA-439,182; EPA-336,731; WO 89/09835; WO 89/12696, and WO 90/01069.

Since the present invention presents the full nucleotide sequence of the genomic BDV nucleotide

sequence, these probes can be identified by comparing this sequence with the sequences of other organisms which may contaminate a test sample. Such comparison can be conducted as described in Example 1 below or using  
5 methods known in the art. The probes preferably contain at least 10 contiguous nucleotides or at least 30 contiguous nucleotides with at least 60% homology along the length of the BDV nucleotide sequence being compared. Examples of such probes and methods for conducting the  
10 PCR for detection are as described in Examples 1 and 2.

#### Assay Kits

The present invention also encompasses immunoassay kits containing BDV antigen(s), preferably each antigen  
15 per container, in a concentration suitable for use in immunoassay. In the kits, the BDV antigens may be bound to a solid support and where needed, the kits may include sample preparation reagents, wash reagents, detection reagents and signal producing reagents.

20 Also included are assay kits for nucleotide hybridization assays which include probes which are specific for BDV or its derivatives. The kits may also include sample preparation reagents, wash reagents, detection reagents and signal producing reagents.

25

#### Therapeutic Uses of Antibodies Directed to BDV proteins and Their Derivatives

Another aspect of the invention presents methods, using antibodies directed to BDV proteins or derivatives,  
30 for treating: (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. Examples of such antibodies are those specific to gp18 and p57. The antibodies may be administered using methods known in the art. Preferably,  
35 this involves passive administration of these antibodies, such as those described in Example 4.

### Peptides Useful For Diagnostics and Therapeutics

Another aspect of the invention presents peptides e.g. the truncated fragments and peptides disclosed in "EXAMPLE 5", below, containing at least one BDV immunoepitope. These peptides can be used in diagnostic assays to detect the presence of a patient's antibodies against BDV. Thus, the peptides are useful for the assays described in the section: "Diagnostic, Prognostic, and Monitoring Uses of BDV proteins and their derivatives". For example, as shown in Example 3 below, recp40, recp23, and recp18 have proved useful for detecting BDV infections. Thus, the epitopes of these recombinant proteins can be mapped, and smaller peptides containing these epitopes and routinely tested for their immunoreactivity with antibodies to BDV, e.g. using the ELISA method shown in Example 3.

*The above peptides can also be used to raise antibodies that may serve as therapeutics against BDV infections such as shown in Example 4 and as described in the section: "Therapeutic Uses of Antibodies Directed to BDV proteins and Their Derivatives".* Examples of methods for synthesizing peptide fragments are described in Stuart and Young in "Solid Phase Peptide Synthesis", 2nd ed., Pierce Chemical Co. (1984). It is contemplated that antibodies which precipitate BDV viral particles would be useful for therapeutic uses. In particular, these antibodies are raised against proteins, and their fragments, expressed on the surface of BDV. It is further contemplated that antibodies against gp18, p57 and their fragments, especially antibodies that precipitate BDV viral proteins would be useful for treating or preventing the disease (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection.

Thus, fragments of BDV proteins, in particular gp18 and p57 and their fragments, can be made starting from either end of their C-termini and NH<sub>2</sub>-termini. For



example, these fragments can be tested according to the ELISA method shown in Example 3 against, *e.g.* sera from horses, rats, or human patients infected with BDV. The fragments that react with the sera would be useful for  
5 detecting the disease and would be useful for raising therapeutic antibodies to treat the disease. Since different animals may react to different epitopes of BDV proteins, one may even tailor the screening test by using the serum from the same species of animal for which one  
10 seeks to develop an assay or therapeutic. For example, if one is seeking a diagnostic test or therapeutic for humans, the sera tested will be preferably that from human patients. Included in this invention are other methods, known in the art, for identifying the  
15 immunoreactive epitopes of a protein and raising antibodies thereto. Further, since antibodies which are immunoreactive with BDV protein may also be found in the sera of patients with neurologic and neuropsychiatric disease not necessarily due to BDV infection, the above  
20 peptides and antibodies raised thereto may also find usefulness in diagnosing, monitoring and treating these patients. Additionally, these peptides may be identified by their immunoreactivity with sera from patients suffering from neurologic and neuropsychiatric disease  
25 not due to BDV infection. Thus, as described in this application, the disease, patient sera to be tested, the diagnostic, monitoring and therapeutic uses are not limited to BDV, and include (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric  
30 disease not due to BDV infection. Further, one can screen for therapeutic ligands or chemicals which bind these peptides. These therapeutic chemicals then may be tested for their therapeutic effect against the above diseases. Other ligands or chemicals which bind the  
35 therapeutic ligands or chemicals can be tested for their ability to bind patients' antisera or antibodies and are thus useful as diagnostics for the diseases.

Preferably, the above peptides and antibodies are also respectively tested for their crossreactivity with antibodies raised by and proteins from organisms unrelated to the above diseases but commonly found in the test sample (e.g. patient's biological sample). Peptides and antibodies that are highly non-specific are preferably not used. To obtain peptides of high specificity, one may also compare the amino acid sequence of BDV protein with that of known contaminating proteins in the test sample. The fragments that are unique, or relatively so, to BDV are then chosen for further screening as described above, e.g. for immunoreactivity with patient's test sample. These comparison can also be done on the nucleotide sequence level.

15

Method for Producing Antibodies to BDV and its Derivatives

Besides whole immunoglobulins, antibodies herein include antigen binding fragments of the immunoglobulins. Examples of these fragments are Fab, F(ab')<sub>2</sub> and Fv. Such fragments can be produced by known methods. Unless otherwise indicated, antibodies herein also include: polyclonal and monoclonal antibodies, monospecific antibodies, and antisera which includes monospecific antisera.

Antibodies to BDV proteins and their derivatives can be produced using standard procedures known in the art. For example, antibodies can be produced by inoculating a host animal such as a rabbit, rat, goat, mouse, etc., with BDV proteins and their derivatives. Before inoculation, the polypeptides or fragments may be first conjugated with keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). After an appropriate time period for the animal to produce antibodies to the polypeptides or fragments, the anti-serum of the animal is collected and the polyclonal antibodies separated from the anti-serum using techniques known in the art. Monoclonal

antibodies can be produced by the method described in Kohler and Milstein (*Nature*, 256:495-497, 1975) by immortalizing spleen cells from an animal inoculated with the polypeptides or fragments thereof. The  
5 immortalization of the spleen cell is usually conducted by fusing the cell with an immortal cell line, for example, a myeloma cell line, of the same or different species as the inoculated animal. The immortalized fused cell can then be cloned and the cell screened for  
10 production of the desired antibody.

The antibodies may also be recombinant monoclonal antibodies produced according to the methods disclosed in Reading, United States Patent No. 4,474,893, or Cabilly et al., United States Patent No. 4,816,567. The  
15 antibodies may also be chemically constructed according to the method disclosed in Segel et al., United States Patent No. 4,676,980.

While the invention is demonstrated using mouse monoclonal antibodies and rat monospecific antisera, the  
20 invention is not so limited. In fact, human antibodies may be used and may prove to be preferable. The latter is especially so if the antibodies are used as therapeutics for humans, as there would be less immunorejection from the human patients receiving these  
25 antibodies. Such antibodies can be obtained by using human hybridomas {Cote et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985)}. In fact, according to the invention, techniques developed for the production of chimeric antibodies {Morrison et al., *Proc.*  
30 *Natl. Acad. Sci.*, 81:6851 (1984); Neuberger et al., *Nature*, 312: 604 (1984); Takeda et al., *Nature*, 314: 452 (1985)} by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate  
35 biological activity (such as ability to activate human complement and mediate antibody-dependent cell-mediated

cytotoxicity) can be used; such antibodies are within the scope of this invention.

#### VACCINE

5 By providing the nucleotide and amino acid sequences of the BDV genome and BDV proteins, respectively, this application enables the production of recombinant BDV {*e.g.* using the technique shown in Schnell, M. J., EMBO J., 13: 4195-4203 (1994)} which can then be attenuated, *e.g.* by  
10 mutagenesis, heat or formaldehyde treatment, to be used as vaccine against (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection. BDV sequences, their mutagenized sequences or fragments thereof, may be  
15 administered, *e.g.* by direct injection, or incorporated into a vector and administered *e.g.* by direct injection, into patients. Examples of the fragments are the truncated fragments and peptides disclosed in "EXAMPLE 5", below. The injections may be by means of a gene gun.  
20 gp18, p57, pol, and proteins produced by the mutagenized or fragmented sequences may also serve as vaccines. Proteinaceous vaccines may be delivered orally, intravenously, intraperitoneally, or intramuscularly. The vaccine may also be contained in a physiologically  
25 compatible solution.

#### BDV Viral Vector Based Delivery System

Another aspect of the invention presents: (A) a BDV-mediated gene transfer for the incorporation and  
30 expression of eukaryotic or prokaryotic foreign genes into another eukaryotic or prokaryotic host; and (B) an *in vitro* BDV-mediated delivery of gene(s) or chemical(s) to a target cell.

In Method A, one or more desired genes are inserted  
35 into the BDV viral vector. The desired gene transfer can be achieved through *in vitro* transfection of a cell or cell

line by the resulting BDV viral vector. The transfected cell or cell line thus expresses the gene(s) of interest and the expression product(s) are harvested. Alternatively, the transfected cell or cell line is later  
5 transplanted into a host, *e.g.* an animal such as a human, in need of the gene product(s). In this case, the gene(s) is expressed *in vivo*. The generation of infectious non-segmented, neurotropic, negative-stranded RNA virus entirely from cloned cDNA, has been described in the case  
10 of rabies virus {Schnell, M. J., et al., EMBO J., 13(18): 4195-4203 (1994)}. The insertion of foreign gene(s) into the BDV viral vector is based on prior art teachings for other viral vectors, which may include insertion of promoters or regulators to control expression of the  
15 foreign gene(s). The transfection and gene therapy is similarly based on prior art teaching for viral vectors.

Such teachings abound, see *e.g.*, U.S. Patent No. 5,219,740 to Miller et al., Jun 15, 1993; U.S. Patent No. 5,256,553 to Overell, Oct. 26, 1993; and WO 91/12329,  
20 assigned to Board of Regents, the University of Texas System, international publication date, Aug. 22, 1991.

Method B utilizes the unique tropism of BDV for specific regions and cells of the nervous systems, *e.g.* neural cells. Thus, BDV vector can be used for *in vivo*  
25 delivery of chemicals or desired genes to these regions. For example, infectious recombinant BDV containing the gene of interest can be used to infect the specific target cells of BDV in a host animal. The host can be a human suffering from deficiency, lack of, or a  
30 malfunctioning of the gene product. The general gene therapy methods can be based on prior art teaching *e.g.* the references cited for Method A, such as WO 91/12329.

In the case of BDV viral vectors, these genes can be those responsible for the survival, proliferation, and  
35 proper functioning of the nervous system. For example, in neurodegenerative diseases, the cells in the patients'

nervous system suffer premature death, and these cells are not regenerated, eventually causing the patients to die. The inserted gene(s) may supplement or replace the dysfunctional gene(s) in these patients to provide gene product(s) necessary for continued survival and proliferation of these cells. Examples of the inserted genes include genes coding for: neurotransmitters, cytokines, growth factors, receptors for the foregoing, enzymes for activation of therapeutic drugs administered to the patients.

Alternatively, the viral vector may contain a nucleotide sequence coding for a toxin. These vectors would infect the host's cells *in vivo*, express the toxin and kill the infected cells. The targeted cells are preferably neoplastic cells, or cells infected by or harboring pathogenic organisms. The vector is preferably further designed to selectively target these cells over normal cells. One means to target the desired cells is by localized injection of the recombinant virus, containing the desired gene, near the target of interest. However, for BDV based gene therapy, the vector or recombinant virus may be delivered peripherally, *i.e.* into subcutaneous tissue, peripheral nerve, or intramuscularly. The neurotropism of the recombinant virus allows it to migrate towards cells of the nervous system to transfect or infect them.

The BDV viral vector is an especially good vehicle for gene therapy and *in vivo* chemical delivery. It has several advantages over the viral vectors known in the art, the most common of which are retroviral vectors. Retroviral vectors require replication of its host cells for transfection. Therefore, retroviral vectors can only be used with dividing/mitotic cells. In contrast, BDV vectors are autonomous, self-replicating vectors and thus can transfect both dividing and non-dividing cells. Thus, BDV is particularly effective for transfecting

nerve cells that normally do not divide and for which BDV is tropic.

Further, BDV does not have a latent stage in its lifecycle, after transfecting a host cell. It thus will  
5 continue to express the desired gene once it has transfecting a cell. This is unlike some viral vectors currently used in the art, such as the herpes viral vector that may enter a latent stage after transfection and thus not express the desired gene product in the  
10 transfecting cell. BDV is also unique in that it is a slow growing virus and is not lytic. Thus, chances of the virus lysing and killing the host cells are nonexistent.

As a further safeguard, the BDV viral vectors may be  
15 made infective but replication-defective, rendering them useful vectors which are unable to produce infective virus, following introduction into a cell. For initiation of productive infection of BDV, a nucleocapsid containing BDV genomic RNA is required, from which  
20 primary transcription of mRNAs and ensuing autonomous and regulated expression of all BDV proteins occurs. Thus, to render the viral vector replication-defective, one may mutate the nucleocapsid protein produced by recombinant virus to prevent encapsidation of newly synthesized  
25 genomic RNA. Additionally, the host cell should preferably be devoid of infectious helper virus which may assist in replication of the BDV. Further, unlike retroviruses and herpes viruses, BDV does not cause disease in and of itself. The deleterious effect of BDV  
30 infection is actually caused by the host's immune-mediated rejection of BDV and BDV antigen expressed on infected cells. The rejection involves cellular immune response which activates the host's effector lymphocytes which then kill the transfecting cells. Antibodies appear  
35 not to be as important in the host's immune response. Thus, one means to avoid Borna disease is to interfere with, avoid, or suppress the host's ability to recognize

or mount an immune response to BDV infected cells. For example, immune response in the host is triggered when T lymphocytes recognize a complex of major histocompatibility complex (MHC) and foreign antigen (in this case, BDV proteins) expressed on the host cell's surface. Thus, to reduce the host's immune response, one may choose to interfere with or prevent the expression of MHC on the transfected cells. This may be achieved by inserting, into the BDV viral vector, a nucleotide sequence which codes for a mRNA (i.e. an antisense mRNA) which would bind the mRNA coding for the component of MHC ("mRNA<sub>MHC</sub>") and prevent the translation and expression of MHC in the transfected cell. Absent MHC, the BDV antigens will not be presented on the host cell surface to trigger immune-mediated rejection in the host. Alternatively, other methods known in the art may be used to avoid the immune rejection of BDV transfected cells.

#### EXAMPLE 1

##### 20 Cloning and Sequencing of Genomic RNA from Borna Disease Virus (BDV) Particles

The studies in this example and Example 2, except with regard to p57, are also described in Briese, T., et al., *Proc. Natl. Acad. Sci. USA*, 91:4362-4366 (1994) and Kliche, S., et al., *J. Virol.*, 68: 6918-6923 (1994), respectively, both of which are hereby incorporated by reference in their entirety. In this example, the BDV genome was cloned to reveal antisense information for five open reading frames (ORFs). From 5' to 3' on the antigenome, the ORFs are p40, p23, gp18, p57 and pol. Proteins p40, p23 and gp18 have been identified in infected cells and tissues: p40 and p23 are expressed at high levels *in vitro* and *in vivo* and are found in the nucleus and cytoplasm of infected cells {Bause-Niedrig, I., M. et al., *Vet. Immunol. Immunopathol.*, 31:361-369 (1992)}. gp 18 is a membrane-



associated glycoprotein that is expressed at lower levels. gp18 was characterized in Example 2 below.

Messenger RNAs {Kliche, S., et al., *J. Virol.*, 68: 6918-6923 (1994); Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990); McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992); Pyper, J. M., et al., *Virology*, 195:229-238 (1993); Thibault, K. J., M.S. thesis; University of California, Irvine (1992); Thierer, J., et al., *J. Gen. Virol.*, 73:413-416 (1992) and VandeWoude, S., et al., *Science*, 250:1278-1281 (1990)} and proteins {Bause-Niedrig, I., et al., *Vet. Immunol. Immunopathol.*, 31:361-369 (1992); Haas, B., et al., *J. Gen. Virol.*, 67:235-241 (1986); Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988); Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985) and Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)} corresponding to three of these ORFs, p40, p23 and gp18, have been found in infected cells and tissues in a 5'-3' expression gradient (p40 > p23 > gp18) {Briese, T., et al., *Proc. Natl. Acad. Sci. USA*: 91:4362-4366 (1994); Cubitt, B., et al., *J. Virol.*, 68:1382-1396 (1994); and Richt, J. A., et al., *J. Gen. Virol.*, 72:2251-2255 (1991)}.

Though Cubitt, B., et al., *J. Virol.*, 68:1382-1396 (1994) purported to have sequenced the BDV genome, their paper contains numerous errors. The errors included (1) failure to recognize deletions in subgenomic RNAs due to splicing; (2) misplacement of ORFs leading to the prediction of a 40kD protein instead of a 57kD protein and failure to detect ORF overlap of p57 with gp18 and pol; and (3) selection of incorrect motifs for initiation of transcription. These mistakes were implicitly acknowledged in a subsequent paper, de la Torre, J. C., *J. Virol.*, 68:7669-7675 (1994). Figure 1 of the latter paper incorporated the correct genomic organization and transcription map described in Example 1 of this application. A later minireview which compares the

sequence differences between the above Cubitt, et al.'s genomic sequence and the sequence described in Example 1 below concludes that the differences seem most likely due to cloning and/or sequencing errors (of Cubitt et al.'s) rather than natural differences between the nucleotide sequences of different strains. Schneeman, A., et al., to be published in *Virology*, 209 (1995); a co-author of the paper is Dr. Robert A. Lamb, the editor-in-chief of *Virology* and a Howard Hughes Medical Institute Investigator. Dr. Lamb was not a collaborator in the work described in Example 1 below..

In this Example, the 8,910 nucleotide BDV viral genome was cloned and sequenced using RNA from BDV particles. The viral genome has complementary 3' and 5' termini and contains antisense information for five open reading frames. Homology to Filo-, Paramyxo- and Rhabdoviridae is found in both cistronic and extracistronic regions. Northern analysis indicates that the virus transcribes mono- and polycistronic RNAs and uses termination/polyadenylation signals reminiscent of those observed in other negative-strand RNA viruses. BDV is likely to represent a previously unrecognized genus, bornaviruses, or family, Bornaviridae, within the order Mononegavirales.

25

#### **MATERIALS AND METHODS**

##### **BDV cDNA Library Preparation and Screening.**

Genomic RNA template for library construction was obtained from an oligodendrocyte cell line (Oligo/TL) acutely infected with BDV Strain V {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 89:11486-11489 (1992)}. For the first genomic library, RNA from one viral particle preparation was polyadenylated with poly(A) polymerase (GibcoBRL, Life Technologies, Inc., Grand Island, New York) to facilitate cloning from the 3' terminus by oligo d(T) primed cDNA synthesis. Libraries were prepared in pSPORT using the SuperScript Plasmid system (GibcoBRL, Life

Technologies, Inc., Grand Island, New York). The first library was screened using pAB5 and pAF4 radiolabeled restriction fragments {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA* 87:4184-4188 (1990)}. Subsequent libraries were screened using radiolabeled restriction fragments from locations progressively 5' on the genomic RNA. 5'-terminal sequence from each library was used to design an oligonucleotide primer for construction of the next library. DNA sequencing and sequence analysis. Plasmid DNA was sequenced on both strands by the dideoxynucleotide chain termination method {Sanger, F., et al., *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)} using bacteriophage T7 DNA polymerase (Sequenase version 2.0; United States Biochemical, Cleveland, Ohio). Five to ten independent clones from each library were sequenced with overlap so that each region of the genomic RNA was covered by at least two clones. Four libraries were analyzed yielding ~8.9 kb of continuous sequence. Nucleic acid sequence was analyzed using the Sequence Analysis Software Package (Genetics Computer, Inc., Madison, Wisconsin). Database searches for related sequences and multiple sequence alignments were performed using FastA and Pileup.

**Sequence Determination at the 3' and 5' Termini of BDV Genomic RNA.** Genomic RNA from one viral particle preparation ( $1-2 \times 10^8$  cells) was treated with tobacco acid pyrophosphatase (Epicentre Technologies, Madison, Wisconsin) and circularized with T4 RNA ligase (New England Biolabs, Inc., Beverly, Massachusetts) {Mandl, C. W., et al., *BioTechniques* 10:484-486 (1991)}. The ligated RNA was reverse transcribed with Superscript II (Gibco BRL, Life Technologies, Inc., Grand Island, New York) using primer 5'-GCCTCCCCTTAGCGACACCCTGTA (SEQ ID NO: 11), complementary to a region 465 nucleotides (nt) from the 5' terminus of the BDV genome. A 2  $\mu$ l aliquot of the reverse transcription reaction was used to amplify the ligated region by the polymerase chain reaction (PCR)

using Stoffel fragment (Perkin-Elmer Cetus, Norwalk, Connecticut). Primers used in the first round of PCR were 5'-GCCTCCCCTTAGCGACACCCTGTA (SEQ ID NO: 11) and 5'-GAAACATATCGCGCCGTGCA (SEQ ID NO: 12), located 241 nt from the 3' terminus of the BDV genome. Amplified products were subjected to a second round of PCR using a nested set of primers: 5'-TACGTTGGAGTTGTTAGGAAGC (SEQ ID NO: 13), 251 nt from the 5' terminus, and 5'-GAGCTTAGGGAGGCTCGCTG (SEQ ID NO: 14), 120 nt from the 3' terminus. PCR products were cloned {Schneider, P. A., et al., *J. Virol.* 68:63-68 (1994)} and sequence across the 5'/3' junction was determined from five independent isolates.

**Northern hybridization.** Poly(A)<sup>+</sup> enriched RNA extracted from acutely infected rat brain using FastTrack (Invitrogen Corp., San Diego, California) was size-fractionated on 0.22 M formaldehyde/1.0% agarose gels {Tsang, S. S., et al., *BioTechniques* 14:380-381 (1993)}, transferred to Zeta-Probe GT nylon membranes (Bio-Rad Laboratories, Richmond, California) and hybridized with random-primed 32P-labeled restriction fragments {Feinberg, A. P., et al., *Anal. Biochem.* 132:6-13 (1983)} representing ORFs across the BDV genome (FIG. 6 b). RNA transfer, hybridization and washing were performed following the manufacturer's protocol (Bio-Rad Laboratories, Richmond, California).

## RESULTS

The following figures present some of the results:

FIG. 3. (a) Organization of the BDV genome. Hatched boxes represent coding sequence complementary to ORFs for identified proteins, p40, p23, gp18, or putative proteins, p57, p180. (p180 is also referred to as pol.) Overlap is indicated by cross-hatched areas. Length of coding sequence corresponding to ORFs in nucleotides is indicated in brackets. Underlined italic numbers indicate length of sequence from stop codon complement to

last templated uridine of termination/polyadenylation signal (black boxes). Italics with arrow indicate number of nucleotides in intervening sequence between p40 polyadenylation signal and p23 coding sequence and  
 5 between p23 polyadenylation signal and gp18 coding sequence, respectively. Italics with dashed arrow indicate number of noncoding nucleotides at termini of the genome. (b) Coding potential of genome. Genomic sequence was translated in all six possible reading  
 10 frames (3'-5' negative sense; 5'-3' positive sense) by using FRAMES (Genetics Computer Group). ORFs are indicated by bars and hatched boxes.

FIG. 4. Alignment of the p180 (pol) ORF and negative-strand RNA virus L-polymerase amino acid  
 15 sequences with PILEUP. Solid lines indicate conserved L-polymerase motifs (a, A, B, C, D). BDV sequence is indicated with double arrowheads. Rhabdoviridae: RaV, rabies virus; VSV, vesicular stomatitis virus; SYN, sonchus yellow net virus. Paramyxoviridae: MeV, measles  
 20 virus; SeV, Sendai virus; NDV, Newcastle disease virus; RSV, respiratory syncytial virus. Filoviridae: MaV, Marburg virus. Numbers indicate amino acid range shown. Uppercase letters in viral sequence lines indicate residues conserved in more than six sequences. Uppercase  
 25 letters in consensus line (Con) indicate presence of identical or conserved amino acids in BDV. Agreement of BDV sequence with either rhabdo- or paramyxoviruses is indicated by \* or x, respectively. +, Nonconserved glycine residue in BDV.

30 FIG. 5. Sequence analysis of BDV genomic termini. (a) Similarity of 3'-terminal BDV sequence to leader regions of Rhabdoviridae (RaV), VSV), Paramyxoviridae (MeV, SeV, NDV, RSV), and Filoviridae (MaV). Abbreviations are as in FIG. 2. EboV, Ebola virus.  
 35 Sequences are aligned by using arbitrary gap insertion to optimize nucleotide matching. (b) Comparison of complementarity at 3' and 5' termini of BDV genomic RNA

with that of four other nonsegmented, negative-strand RNA viruses. The 3' and 5' terminal sequences for each virus are shown in viral RNA (3'-5', negative sense) orientation. Underlined sequence refers to

5 transcriptional start of first gene or end of the L-polymerase gene (also referred to as "pol gene"), respectively (predicted for BDV). The end of the L-polymerase gene of RaV is located outside the region shown.

10 FIG. 6. Map of BDV subgenomic RNAs relative to the viral antigenome. (a) Northern hybridization analysis of rat brain poly(A)<sup>+</sup> RNA. Each lane was hybridized with a probe representing a major BDV ORF as indicated by the letters A-E (see b). Results of hybridization with  
15 probes C\* and E\* were identical to results of hybridization with probes C and E, respectively (data not shown). Numbers at left indicate size of RNA markers in kilobases. Numbers at right indicate estimated size of major transcripts. (b) Position of viral transcripts  
20 with respect to antigenome as determined by Northern hybridization and sequence analysis. Dashed lines indicate regions in the 1.5-kb RNA and the 6.1-kb RNA that contain a deletion. The boundaries of the deletions are not known. Relative positions of probes used for  
25 Northern hybridization are shown. On the ORF map, potential start codons are indicated with upward lines; ◇, start codons predicted to be functional; x, potential start codon present in strain V that is absent in strain He/80 (see text). Potential termination sites are  
30 indicated with downward lines. Use of T2 and T3 has been confirmed {McClure, M. *et al.*, *J. Virol.*, 66:6572-6577; Thierer, J. *et al.*, *J. Gen. Virol.*, 73:413-416}; use of T5 and T7 is consistent with hybridization results. Termination at t1, t4 and t6 has not been observed (see a). (c)  
35 Alignment of the seven potential termination sites of BDV. Location of sites is indicated in the ORF map. Stop codons are underlined. Lowercase letters indicate

termination/polyadenylation consensus sequence. No termination/polyadenylation site was found at or near the end of the gp18 ORF.

#### 5 Sequencing of Genomic BDV RNA.

Beginning from the 3' terminus, a series of four overlapping cDNA libraries was constructed using BDV particle RNA {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 89:11486-11489 (1992)} as template. Previous studies  
10 have shown that the genomic RNA is not polyadenylated {de la Torre, J., et al., *Virology* 179:853-856 (1990)}. Thus, to construct the first library, genomic RNA was polyadenylated *in vitro* in order to facilitate oligo d(T)-primed cDNA synthesis. For the subsequent three  
15 libraries, genome-complementary oligonucleotide primers were designed based on 5' terminal sequence determined in the previous round of cloning. Each region of the genome was sequenced using a minimum of two independent clones. To determine the sequences at the termini, genomic RNA  
20 was circularized and sequenced across the junction using five independent clones.

The 8,910 nt BDV genome contained antisense information for five major ORFs flanked by 53 nt of noncoding sequence at the 3' terminus and 91 nt of  
25 noncoding sequence at the 5' terminus (FIG. 3). In 3'-5' order, the first two ORFs encoded two previously described viral proteins, p40 {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992)} and p23 {Thierier, J., et al., *J. Gen. Virol.* 73:413-416 (1992)}. The third, fourth and  
30 fifth ORF had coding capacities of 16 kDa (gp18), 57 kDa (p57) and 190kDa (p180), respectively (FIG. 3a). Note: p180 is now known as "pol". Predicted amino acid sequence for the 16 kDa ORF correlated with microsequence data for an 18 kDa BDV glycoprotein (see the section  
35 below for gp18 glycoprotein), originally described as the Borna disease-associated 14.5 kDa protein {Schädler, R.,

et al., *J. Gen. Virol.* 66:2479-2484 (1985)}. The first three ORFs showed no overlap and were in frame with the fifth ORF (FIG. 3b). The 57 kDa ORF was in a +1/-2 frame relative to the other four ORFs and overlapped the adjacent ORF for gp18 by 28 amino acids and ORF p180 by 34 amino acids. All ORFs were located on the (+) strand, complementary to the genomic RNA. ORF analysis of the genomic (-) strand showed only three small ORF's, each with a coding capacity of less than 16 kDa (FIG. 3b).

10

#### Homology analysis of coding sequence.

Predicted amino acid sequence for the identified ORFs was used to examine databases for similarity to other proteins. Previous analysis of the ORF encoding p40 had revealed distant sequence similarity to L-proteins of Paramyxoviridae and Rhabdoviridae {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992)}. FastA analysis of translated sequence from ORFs p23, gp18 and p57 showed no apparent similarity to other viral sequences; however, ORF p180 sequence consistently retrieved L-polymerases of Paramyxo- and Rhabdoviridae. Alignment of ORF p180 (pol) sequence with sequence of RNA-dependent RNA polymerases of negative-strand RNA viruses showed conservation of both sequence and linear order of regions homologous among these proteins. Extensive conservation was found in the four characteristic motifs for L-polymerases of negative-strand RNA viruses (A-D in FIG. 4) {Poch, O., et al., *EMBO J.* 8:3867-3874 (1989) and Poch, O., et al., *J. Gen. Virol.* 71:1153-1162 (1990)}. With the exception of the glycine residue in motif B (position 322 of the alignment), conservation was found for the individual amino acid residues postulated to participate in polymerase function {Poch, O., et al., *EMBO J.* 8:3867-3874 (1989)}. Conservation was also found for a motif (a in FIG. 4) proposed to participate in template recognition



{Poch, O., et al., *J. Gen. Virol.* 71:1153-1162 (1990) and Barik, S., et al., *Virology* 175:332-337 (1990)}. The GCG/pileup alignment placed ORF p180 sequence between polymerases of Paramyxo- and Rhabdoviridae. This intermediate position is reflected by the presence of conserved amino acids which are in agreement with either the rhabdo- or the paramyxovirus sequences (\* or x, respectively; FIG. 4). The distance between conserved motifs a and A was found to be short in BDV as it is in rhabdoviruses, whereas this region is highly variable in length and sequence among paramyxoviruses {Poch, O., et al., *J. Gen. Virol.* 71:1153-1162 (1990)}. The GCG/pileup generated dendrogram, obtained using complete ORF p180 and L-protein sequences, indicated that the putative BDV polymerase was more closely related to L-polymerases of Rhabdoviridae than Paramyxoviridae.

#### Analysis of Noncoding Sequence at the Genomic Termini.

3' terminal genomic sequence had a high A/U content of 60.5 % with an A to U ratio of ~1:2, similar to 3' leader sequences of other negative-strand RNA viruses. At the extreme 3' end, filo-, paramyxo- and rhabdoviruses have a common G/U rich region (FIG. 5a). In BDV, as in respiratory syncytial virus, rabies virus and filoviruses, this region was not located at the 3' extremity. Comparison of the 3' and 5' termini of BDV genomic RNA revealed complementarity similar to that found in other negative-strand RNA viruses {Keene, J. D., et al., *J. Virol.* 32:167-174 (1979) and Tordo, N., et al., *Virology* 165:565-576 (1988)} (FIG. 5b). Alignment of the genomic termini allowed formation of a terminal panhandle, with the first three nucleotides unpaired. The subsequent complementary area of 6 nucleotides (positions 4-9 and 8907-8902) could be extended by one gap insertion between position 8901/8,902 resulting in an

additional 10 nt stretch of complementarity with a single mismatch (positions 18 and 8994; FIG. 5b).

#### Identification of Potential Termination/ Polyadenylation

##### 5 Sites.

Sequence preceding the poly(A) tracts of two cloned BDV mRNAs (UA<sub>5</sub>) {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992) and Thierer, J., et al., *J. Gen. Virol.* 73:413-416 (1992)} was used to analyze genomic sequence for homologous sites that could serve as potential termination/ polyadenylation signals. Seven sites were found (FIG. 6 c). Northern hybridization experiments supported use of four of these sites (T2, T3, T5 and T7) and allowed identification of a termination/polyadenylation signal consensus sequence (CMNMYM<sub>2</sub>NWA<sub>6</sub>), where M is A or C, Y is C or U, and W is A or U. Only one of the three remaining sites (t6) matched the consensus sequence (FIG. 6c).

##### 20 Northern Hybridization Analysis.

Restriction fragments representing the five ORFs were used as probes for hybridization to poly(A)<sup>+</sup> enriched RNA isolated from acutely infected rat brain by FastTrack (FIG. 6a and b). Because this procedure does not entirely eliminate poly(A)<sup>+</sup> RNAs, small levels of BDV genome-size RNA can usually be detected in these preparations. To allow determination of the relative abundance of RNAs detected by each probe, exposure times were normalized to the signal of the 8.9-kb RNA. Consistent with the 3' to 5' transcriptional gradient found for other negative-strand RNA viruses, of the eight subgenomic RNAs identified, those detected by the 3'-most probes (genomic orientation), A and B, were more abundant than those detected by the more 5' probes (FIG. 6a and b).

Mapping of the eight transcripts to the genome by Northern hybridization indicated use of only three sites

for transcriptional initiation and four sites for termination. Probes C\* and E\* were used to distinguish between termination at T5 or t6 (FIG. 6b). The patterns of hybridization with probes C\* and E\* were identical to those obtained with probes C and E, respectively indicating termination at T5 (data not shown). Probes corresponding to p40 (A) and p23 (B) detected monocistronic RNAs of 1.2 kb and 0.75 kb, respectively (FIG. 6). Probes A and B also detected a 1.9 kb RNA consistent with failure of transcriptional termination at the p40 termination site {Pyper, J. M., et al., *Virology* 195:229-238 (1993)}. Transcriptional readthrough was also found for polycistronic transcripts of 3.5, 2.8 kb and 7.1 kb. The 3.5 kb RNA detected by probes B, C, D and C\*, is likely to initiate at or near the beginning of ORF p23 and terminate at T5. The 2.8 kb RNA detected by probes C, D and C\*, is likely to initiate at or near the beginning of ORF gp18 and terminate at T5. The 7.1 kb detected by probes C, D, C\*, E\* and E, is likely to initiate at or near the beginning of ORF gp18 and to continue through T5 until it terminates at T7. Probes C and C\* both hybridized to a 1.5 kb RNA and a 6.1 kb RNA. Interestingly, neither the 1.5 kb RNA nor the 6.1 kb RNAs was detected by probe D, located between C and C\* on the viral genome. These findings are consistent with posttranscriptional modification resulting in a 1-1.3 kb deletion (FIG. 6).

#### DISCUSSION

The order Mononegavirales, which incorporates the families Filoviridae, Paramyxoviridae and Rhabdoviridae, has distinct characteristics that include: (1) a nonsegmented negative sense RNA genome, (2) linear genome organization in the order 3' untranslated region /core protein genes /envelope protein genes /polymerase gene /untranslated 5' region, (3) a virion associated RNA-dependent RNA polymerase, (4) a helical nucleocapsid

that serves as template for replication and transcription, (5) transcription of 5-10 discrete, unprocessed mRNAs by sequential interrupted synthesis from a single promoter and (6) replication by synthesis of a positive sense antigenome {Pringle, C. R., et al., *Arch. Virol.* 117:137-140 (1991)}. The genomes of rhabdo-, paramyxo- and filoviruses range in size from 11 to 20 kb. The BDV genome has been estimated to be between 8.5 {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA* 87:4184-4188 (1990) and de la Torre, J., et al., *Virology* 179:853-856 (1990)} and 10.5 kb {VandeWoude, S., et al., *Science* 250:1276-1281 (1990) and Richt, J., et al., *J. Gen. Virol.* 72:2251-2255 (1991)} in length. Our data confirm that the BDV genome, at only 8910 nt, is smaller than those of other negative-strand RNA viruses. Several features suggest that BDV is a member of the order Mononegavirales: organization of ORFs on the genome, extensive sequence similarities of the largest BDV ORF to L-polymerases of rhabdo-, paramyxo- and filoviruses, homology of 3' noncoding sequence to leader sequences of Mononegavirales and complementarity of BDV genomic termini.

In 5' to 3' antigenomic orientation, the first ORF contains 1110 nt. Due to a more favorable translation initiation context {Kozak, M., *Nucleic Acids Res.* 15:8125-8148 (1987)}, it is likely that the second AUG codon, 39 nt inside the ORF, is used to express a 357 aa protein of 39.5 kDa (p40) {Pyper, J. M., et al., *Virology* 195:229-238 (1993)}. 26 nt downstream of the stop codon is a polyadenylation signal {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992)} (T2, FIG. 6 b and c). The second ORF starts 79 nt from the p40 polyadenylation site. It has a length of 603 nt coding for a 201 aa protein of 22.5 kDa (p23). The stop codon of ORF p23 is part of the polyadenylation signal {Thierier, J., et al., *J. Gen. Virol.* 73:413-416 (1992)} (T3, FIG. 6b and c). Analysis of the

intergenic region between ORFs p40 and p23 has shown that this sequence is less conserved among different BDV isolates than coding sequences for p40 and p23 {Schneider, P. A., et al., *J. Virol.* 68:63-68 (1994)}.

5 Therefore, expression of a small ORF in this region (x, FIG. 3 b); {VandeWoude, S., et al., *Science* 250:1276-1281 (1990) and Pyper, J. M., et al., *Virology* 195:229-238 (1993)} that overlaps with ORF p23 seems unlikely {Schneider, P. A., et al., *J. Virol.* 68:63-68 (1994)}. Ten  
10 nt downstream of the p23 polyadenylation signal is the third ORF, 426 nt in length, that codes for a 142 aa (16.2 kDa) protein. Due to glycosylation, the protein expressed from this ORF has a Mr of ~18 kDa (gp18).

No polyadenylation signal similar to those  
15 identified for p40 and p23 mRNAs {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992) and Thierer, J., et al., *J. Gen. Virol.* 73:413-416 (1992)} was found near the end of the gp18 ORF (FIG. 6b and c). Instead, the following ORF overlaps with the end of the gp18 ORF by 28 aa. It has  
20 a total size of 1,509 nt that could code for a 503 aa protein of 56.7 kDa (p57). The ORF has two AUG codons in the overlap with gp18. A third AUG located outside the overlap is 451 nt from the beginning of the ORF. Which, if any, of these AUGs is used is unknown as no protein  
25 has been identified. A potential polyadenylation site is located 28 nt downstream of the p57 ORF (t4). However, Northern hybridization results suggest that this site is a weak or nonfunctional signal, because no major transcript(s) were found to stop at this position (FIG.  
30 6).

The fifth ORF encompasses more than half the length of the genome. A potential polyadenylation site (T7), similar to that seen at the end of ORFs p40 and p23, is found 33 nt from the stop codon of p180 (pol) ORF (FIG.  
35 6b and c). Deletions identified by Northern hybridization analysis suggested that viral mRNAs might

undergo post-transcriptional modification by RNA splicing. This hypothesis was subsequently confirmed by applicants {Schneider, P.A. *et al.*, *J. Virol.*, 68:5007-5012 (1994); Schneemann, A. *et al.* *J. Virol.*, 68:6514-6522 (1994),  
5 hereby incorporated in their entirety.} RNA splicing extends the pol ORF by 459 nucleotides allowing prediction of a protein of 190kDa. {Schneider, P. *et al.*, *J. Virol.*, 68:5007-5012 (1994)}. Although functional studies  
10 of BDV proteins have not yet been done, the organization of the viral genome together with the limited biochemical data available suggest possible roles for individual proteins in the virus life cycle. Four lines of evidence suggest that p40 is likely to be a structural protein:  
15 (1) like nucleocapsid proteins (N) of rhabdo- and paramyxoviruses {Banerjee, A. K., *et al.*, *Pharmacol, Ther.* 51:47-70 (1991)} (except pneumoviruses {Collins, P. L., *The Paramyxoviruses*, ed. Kingsbury, D. W. (Plenum, New York), pp. 103-162 (1991)}), p40 is found in the most 3' position on the genome; (2) p40 is similar in size to N  
20 proteins; (3) both p40 {Pyper, J. M., *et al.*, *Virology* 195:229-238 (1993) and Ludwig, H., *et al.*, *Prog. Med. Virol.* 35:107-151 (1988)} and N proteins {Banerjee, A. K., *et al.*, *Pharmacol, Ther.* 51:47-70 (1991)} are abundant in infected cells and particles; (4) neither N proteins  
25 {Banerjee, A. K., *et al.*, *Pharmacol, Ther.* 51:47-70 (1991)} nor p40 {Thiedemann, H., *et al.*, *J. Gen. Virol.* 73:1057-1064 (1992)} are phosphorylated or glycosylated. p23, a phosphorylated protein {Thiedemann, H., *et al.*, *J. Gen. Virol.* 73:1057-1064 (1992)}, is in the next position on the  
30 genome. ORF p23 corresponds in position to genes coding for phosphoproteins in Paramyxoviridae (P) and Rhabdoviridae (NS) {Banerjee, A. K., *et al.*, *Pharmacol, Ther.* 51:47-70 (1991)}. This suggests that p23 might serve a similar role in the BDV system. In support of this  
35 hypothesis, GCG analysis showed that the protein has a

high Ser/Thr content (16%), is charged (pI 4.8) and contains a N-terminal cluster of acidic amino acids compatible with structural features of P/NS proteins {Banerjee, A. K., et al., *Pharmacol, Ther.* 51:47-70 (1991)}.

5 In previously described Mononegavirales, the next gene codes for matrix protein (M) {Banerjee, A. K., et al., *Pharmacol, Ther.* 51:47-70 (1991)}. gp18 occupies this position on the BDV genome. Though small for a matrix protein, gp18 has a predicted pI ,10, that is close to

10 the basic pI of M proteins, ~9, and its membrane-association would be compatible with a matrix protein function. For p57, computer analysis predicted similarities to glycoproteins of negative-strand RNA viruses: potential glycosylation sites as well as

15 N-terminal and C-terminal hydrophobic "anchor" domains (data not shown). The largest ORF (pol) is located most 5' on the genome. Its size, 5' position and conservation of motifs considered critical to L-polymerase activity, suggest that this ORF is likely to code for the BDV

20 polymerase (FIG. 6).

Analysis of Northern hybridization experiments in conjunction with genomic sequence data has allowed construction of a tentative transcription map (FIG. 6). While it has not been possible to identify signals for

25 initiation of transcription by using consensus sequences of other negative-strand RNA viruses, we have identified consensus sequence for termination/polyadenylation in BDV using known ends of p40 and p23 mRNAs {McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992) and Thierer, J., et al.,

30 *J. Gen. Virol.* 73:413-416 (1992)} (FIG. 6c). These sequences appear to function as weak termination signals. Unlike other negative-strand RNA viruses, BDV shows a high frequency of readthrough transcripts. Organization and sequence similarities to Filo-, Paramyxo- and

35 Rhabdoviridae suggest that BDV is a member of the order Mononegavirales. Dependent on the parameters and regions selected for homology analysis, BDV can be represented as

being more closely related to filo-, paramyxo- or rhabdoviruses. Overlap of coding sequence, high frequency of polycistronic readthrough transcripts and posttranscriptional modification are properties of the BDV system not found in other members of the order Mononegavirales. These features could serve as independent mechanisms for modulation of gene expression to achieve the persistent, non-cytopathic infection that is a cardinal characteristic of this neurotropic virus.

10

### EXAMPLE 2

#### BDV Glycoprotein gp18

Using methods for isolation of the 14.5-kDa protein {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}, we have purified a glycoprotein from BDV-infected rat brain that is encoded by a 429-nucleotide (nt) ORF located 3' to ORF p23 on the viral antigenome. The protein is predicted to be 16.2 kDa; glycosylation results in a 1- to 2-kDa increase in molecular weight. This glycoprotein, gp18, is the first glycoprotein to be identified in the BDV system. Lectin binding and endoglycosidase sensitivity assays suggest that gp18 is an unusual N-linked glycoprotein.

### 25 MATERIALS AND METHODS

#### Infection of animals and cultured cells.

Animals and cells were infected with BDV strain He/80 {Herzog, S., et al., *Med. Microbiol. Immunol.*, 168:153-158 (1980) and Schneider, P. et al., *Virol.* 68:63-68 (1994)}. Newborn Lewis rats were infected by intracranial injection with  $1.5 \times 10^4$  focus-forming units of BDV. Three weeks after infection, animals were sacrificed and brains were removed for isolation of BDV particles {Carbone, K., et al., *J. Virol.*, 61:3431-3440 (1987)} or gp18. C6 cells and MDCK cells were persistently infected with BDV as described previously {Carbone, K. M., *J. Virol.*, 67:1453-1460 (1993) and Herzog,



S., et al., *Med Microbiol. Immunol.*, 168:153-158 (1980)}.  
Monolayers of rabbit fetal glial cells were acutely  
infected by adding BDV at 1.0 focus-forming unit per cell  
to the culture medium (Dulbecco modified Eagle medium, 5%  
5 fetal calf serum; Gibco BRL, Grand Island, New York).

#### Protein purification and microsequencing.

Protein was purified from infected cells and tissues  
by detergent-salt extraction by the method of Schädler et  
10 al. {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484  
(1985)}. For microsequencing, protein was cleaved with  
10% cyanogen bromide in 75% formic acid (Sigma Chemical  
Co., St. Louis, Missouri). Peptide fragments were  
separated by reverse-phase high-performance liquid  
15 chromatography (RP-HPLC) on a Vydac C-18 column, using a  
trifluoroacetic acid/acetonitrile gradient. Sequence  
determinations were performed by automated Edman  
degradation on a Hewlett-Packard model G1000A protein  
sequencer.

20

#### Antibodies.

Antibodies to purified gp18 were produced in 3-  
month-old BALB/c mice. Animals were injected  
subcutaneously with 5 µg of protein in Freund's complete  
25 adjuvant and boosted 3 weeks later with a subcutaneous  
injection of 3 µg of protein in Freund's incomplete  
adjuvant. For 6 weeks thereafter, at 2-week intervals,  
animals received intraperitoneal injections of 5 µg of  
protein in phosphate-buffered saline (PBS) with 5 µg of  
30 lipopolysaccharide (*Salmonella typhimurium*; Difco, Detroit,  
Michigan) (three injections). Blood was drawn every 2  
weeks during weeks 7 through 28 for measurement of serum  
antibody titer to purified protein by Western blotting  
(immunoblotting). Antisera collected at week 28 were  
35 used for virus neutralization studies. Rabbit antisera  
to recombinant BDV p40 and p23 were used as controls (see  
Example 3, below).

**Cloning and sequencing of CDNA encoding gp18.**

gp18-specific oligonucleotides were used to amplify full-length coding sequence for gp18 from two BDV-infected adult rat brain cDNA libraries {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990) and McClure, M. A., et al., *J. Virol.* 66:6572-6577 (1992)} as well as total cellular RNA {Chirgwin, J. J., et al., *Biochemistry*, 18:5294-5299 (1979)} and poly(A)<sup>+</sup> RNA {Aviv, H., et al., *Proc. Natl. Acad. Sci. USA*, 69:1408-1412 (1972)} extracted from infected rat brain. Reverse transcription (RT) was performed with an oligo(dT) primer and Superscript II (Gibco BRL, Life Technologies, Inc., Grand Island, New York ). PCR was carried out with Ampli-Taq Stoffel fragment according to standard protocols (Perkin-Elmer, Norwalk, Connecticut) with the following primer pair: 5'-terminal *Xho*I-gp18 sense oligonucleotide (*Xho*I-gp18-S1), TCCTCGAGATGAATTCAAAACATTCCTATC (nt 1892 to 1914; *Xho*I restriction site indicated by underlining) (SEQ ID NO: 15); and 3'-terminal gp18 antisense oligonucleotide (gp18-AS1), CTAAGGCCCTGAAGATCGAAT (nt 2301 to 2321) (SEQ ID NO: 16). Products were purified by agarose gel electrophoresis using a USBioclean purification kit (U.S. Biochemical, Cleveland, Ohio) and cloned into Bluescript SKII+ (Stratagene, San Diego, California) prepared with 3' T overhangs {Marchuk, D., et al., *Nucleic Acid Res.*, 19:1154 (1990)}. A minimum of three independent clones from each template source was sequenced on both strands by the dideoxynucleotide chain termination method using bacteriophage T7 DNA polymerase (Sequenase; U.S. Biochemical, Cleveland, Ohio). The plasmid resulting from amplification of neonatally infected rat brain RNA was named pBDV-gp18.

***In vitro* transcription, translation, and cotranslational processing.**

Plasmid clones pBDV-gp18 and pBDV-23 {Thibault, K. J., M.S. thesis, University of California, Irvine (1992)}  
5 linearized with *EcoRI* were used as templates for *in vitro* synthesis of capped RNA transcripts. Transcription products or *Saccharomyces cerevisiae*  $\alpha$ -factor mRNA (control for glycosylation) were translated *in vitro* by using nuclease-treated rabbit reticulocyte lysates (Promega Corp.,  
10 Madison, Wisconsin) in the presence of [<sup>35</sup>S]methionine (Amersham Corporation, Arlington Heights, Illinois). Cotranslational processing was assessed by *in vitro* translation using reticulocyte lysates supplemented with canine microsomal membranes (Promega, Madison,  
15 Wisconsin). Transcription, translation, and cotranslational processing studies were performed according to the manufacturer's protocols. Translation products were immunoprecipitated with mouse anti-gp18 serum and then size fractionated by sodium dodecyl  
20 sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (13% gel) {Laemmli, U. K., et al., *J. Mol. Biol.*, 80:575-581 (1973)} for autoradiographic analysis. Methods for immunoprecipitation and autoradiography have been described elsewhere {Lipkin, W. I., et al., *Proc. Natl. Acad.*  
25 *Sci. USA*, 87:4184-4188 (1990)}.

**Protein gel electrophoresis and immunoblotting.**

Proteins were size fractionated by SDS-PAGE (12% gel) and then transferred to Immobilon-N membranes  
30 (Millipore Corp., Bedford, Massachusetts). Primary antisera for immunoblotting were from rats chronically infected with BDV (day 100 after intracranial infection) or mice immunized with purified gp18. The secondary antibody was alkaline phosphatase-conjugated goat  
35 antimouse immunoglobulin G (Sigma Chemical Co., St.

Louis, Missouri); the substrate was Western Blue (Promega Corp., Madison, Wisconsin).

#### Carbohydrate analysis.

5 Purified protein was size fractionated by SDS-PAGE (13% gel) and then either silver stained for detection of protein or carbohydrate {Tsai, C. M., et al., *Anal. Biochem.*, 119:115-119 (1982)} or transferred to Immobilon-N membranes (Millipore, Bedford, Massachusetts) for lectin  
10 staining. The carbohydrate composition of immobilized protein was determined by using a DIG Glycan Differentiation Kit (Boehringer Mannheim, Indianapolis, Indiana) and peroxidase-labeled *Bandeiraea simplicifolia* agglutinins I and II (BS-I and BS-II; Sigma Chemical Co.,  
15 St. Louis, Missouri). The substrate for peroxidase was 4-chloro-1-naphthol (Pierce Chemical Company, Rockford, Illinois). Glycosidase digests of native and denatured protein (incubated for 5 minutes at 100°C in 0.01% SDS) were performed according to the manufacturer's protocols,  
20 using the following endoglycosidases: endoglycosidase F and  
N-glycosidase F; O-glycosidase; N-glycosidase F; endoglycosidase F, N-glycosidase free; endoglycosidase H; and endo- $\beta$ -galactosidase (Boehringer Mannheim).

25

#### RESULTS

The following figures present some of the results:

FIG. 7. Sequence of ORF gp18. The diagram shows the location of ORF gp18 on the viral antigenome (5'-3')  
30 relative to ORFs p40 and p23 (boxes). ORF gp18 sequences were from Oligo/TL cells infected with BDV strain V (SV) and rat brain infected with BDV He/80 (RB). Peptide sequences (P#1, P#2, and P#3) were obtained by microsequencing of purified protein from He/80-infected  
35 rat brain. Periods indicate identical nucleotide or amino acid sequences. Variable amino acid residues (large asterisk) and stop codons (small asterisks) are

indicated. Underlining indicates potential glycosylation sites.

FIG. 8. Glycan determination of gp18. gp18 isolated from infected rat brain was size fractionated by SDS-PAGE (12% gel) then transferred to an Immobilon-N membrane for lectin staining (see Materials and Methods). Lanes: 0, protein detection by mouse anti-gp18 serum; 1, ConA; 2, wheat germ agglutinin; 3, *D. stramonium* agglutinin; 4, BS-I; 5, BS-II; 6, *G. nivalis* agglutinin; 7, *S. nigra* agglutinin; 8, *M. amrensis* agglutinin; 9, peanut agglutinin. Positions of molecular weight markers are shown in kilodaltons at the right.

FIG. 9. gp18 is sensitive to endoglycosidases. gp18 isolated from infected rat brain was treated with either buffer alone or endoglycosidase. Protein was size fractionated by SDS-PAGE (13% gel) and detected by silver staining. Lanes: 1, buffer; 2, endoglycosidase F plus *N*-glycosidase F; 3, endoglycosidase F (*N*-glycosidase free); 4, endo- $\beta$ -galactosidase. Positions of molecular weight markers are shown in kilodaltons at the right.

FIG. 10. *In vitro* transcription, translation, and cotranslational processing of gp18. RNA transcripts were synthesized from pBDV-23 (a nonglycosylated BDV protein control) or pBDV-gp18 and translated *in vitro* by using rabbit reticulocyte lysates in either the absence or presence of canine microsomal membranes. [<sup>35</sup>S]methionine-labeled translation products were immunoprecipitated with antisera to p23 or gp18 and protein A-Sepharose and then size fractionated by SDS-PAGE (13% gel) for autoradiography (A) or transferred to Immobilon-N membranes for ConA lectin staining (B). Translated gp18 in lane 5 of panel A and lane 3 of panel B was incubated with endoglycosidase F plus *N*-glycosidase F prior to SDS-PAGE. (A) Lanes: 1, pBDV-23 RNA; 2, pBDV-23 RNA plus microsomal membranes; 3, pBDV-gp18 RNA; 4, pBDV-gp18 RNA plus microsomal membranes; 5, pBDV-gp18 RNA plus

microsomal membranes, incubated with endoglycosidases. The long arrow indicates the position of glycosylated protein (lanes 3 and 4); the short arrow indicates the position of protein after treatment with endoglycosidase F plus *N*-glycosidase F (lane 5). The asterisk indicates nonspecific background signal (lane 5). Positions of molecular weight markers are shown in kilodaltons at the right. (B) Lanes: 1, pBDV-gp18 RNA; 2, pBDV-gp18 RNA plus microsomal membranes; 3, pBDV-gp18 RNA plus microsomal membranes, incubated with endoglycosidases.

#### Isolation of gp18.

Protein was isolated from neonatally infected rat brain, acutely infected rabbit fetal glial cells (two passages), persistently infected C6 cells, and persistently infected MDCK cells, using the method of Schädler et al. {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}. The purity of the protein was confirmed by silver staining of the protein after SDS-PAGE (data not shown). The quantity of protein was estimated in silver-stained gels by using lysozyme standards. Typical yields were 5  $\mu$ g of protein from one neonatally infected rat brain and 2  $\mu$ g of protein from 10<sup>8</sup> infected cultured cells. Protein from neonatally infected rat brain was used for microsequencing, carbohydrate analysis, and immunization of mice.

#### Protein and nucleic acid sequence analysis.

Direct microsequencing of gp18 was not possible because of a blocked amino terminus; thus, to allow analysis, the protein was cleaved with cyanogen bromide. Sequencing of the cleavage mixture indicated the presence of three N termini. From the mixture, two peptides (peptides 1 and 3; FIG. 7) were isolated by RP-HPLC and sequenced individually, allowing inference of a third sequence (peptide 2; FIG. 7) by subtraction. Peptide sequences were used as probes to search ORFs located on

the BDV antigenome. The peptide sequences obtained from the purified gp18 mapped to a 429-nt ORF (ORF gp18) on the viral antigenome that predicts a 142-amino-acid protein with a molecular weight of 16,244 (FIG. 7).

5        Genomic sequence corresponding to the gp18 ORF was used to design probes and primers for identifying mRNA encoding gp18. In each of two cDNA libraries prepared from BDV-infected adult rat brain poly(A)<sup>+</sup> RNA {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990) and  
10    McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992)}, 100,000 recombinants were screened by hybridization with a 271-bp *HincII-HinfI* restriction fragment from pTB-BDV 5.82 (nt 2062 to 2333 in the viral genome) {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 91:4362-4366 (1994)}. These  
15    libraries were also screened by PCR using the 5'-terminal *XhoI*-gp18 sense primer (nt 1892 to 1914) and oligo(dT). Total cellular and poly(A)<sup>+</sup> RNAs extracted from persistently infected C6 cells, BDV-infected adult rat brain, or 3-week-old neonatally infected rat brain (the  
20    peak time point for *in vivo* expression of gp18) were subjected to RT-PCR using oligo(dT) in combination with the 5'-terminal *XhoI*-gp18 sense primer. No gp18-specific transcript corresponding to the size of ORF gp18 was obtained in these experiments. In contrast, use of the  
25    5'-terminal *XhoI*-gp18 sense primer in combination with a 3'-terminal gp18 antisense primer (nt 2301 to 2321) allowed amplification of gp18 sequences from any of these sources by RT-PCR. In spite of variability at the nucleic acid level, the predicted amino acid sequence  
30    obtained from the different sources was the same as for strain V genomic sequence, with the exception of a single exchange in position 108 (E→D) (FIG. 7).

#### Characterization of gp18 as a glycoprotein.

35        Purified gp18 was size fractionated by SDS-PAGE. Modified silver staining revealed the presence of

carbohydrate; thus, fractionated protein was blotted onto Immobilon-N membranes to determine the presence of individual saccharides through lectin binding studies. Binding was observed with *Candavalia ensiformis* agglutinin (ConA), wheat germ agglutinin, *Datura stramonium* agglutinin, BS-I, and BS-II but not with *Galanthus nivalis* agglutinin, *Sambucus nigra* agglutinin, *Maackia amurensis* agglutinin, and peanut agglutinin (FIG. 8). This staining pattern was consistent with the presence of N-acetylglucosamine, N-acetylgalactosamine, mannose, and galactose. In addition, native and denatured proteins were digested with specific endoglycosidases, size fractionated by SDS-PAGE, and then stained to assess molecular weight shift and presence or absence of carbohydrate. Treatment with O-glycosidase or endoglycosidase H had no effect (data not shown). In contrast, treatment with endoglycosidase F and N-glycosidase F resulted in a loss of 1 to 2 kDa (FIG. 9) and abrogation of lectin staining with ConA (data not shown). Treatment with endoglycosidase F (N-glycosidase free) or endo- $\beta$ -galactosidase also resulted in a loss of 1 to 2 kDa (FIG. 9).

***In vitro* transcription, translation, and processing of gp18.**

With linearized pBDV-gp18 used as a template, gp18 RNA was transcribed and translated *in vitro* in either the presence or absence of canine microsomal membranes. The gp18 RNA directed translation of two proteins of 16 and 18 kDa that were recognized by monospecific murine antiserum to purified gp18. Translation in the presence of microsomal membranes led to an increase in the relative proportion of the 18-kDa protein. Treatment with endoglycosidase F resulted in loss of the 18-kDa protein species (FIG. 10A). Glycosylation of the 18-kDa species was also shown by lectin binding studies performed after translation products were size



fractionated by SDS-PAGE and transferred to membranes. The 18-kDa protein was recognized by ConA, whereas the 16-kDa protein did not bind ConA (FIG. 10B). Modification of translated protein by the microsomal  
5 membranes was specific for gp18. Translation of RNA encoding BDV p23, which encodes a potential N-glycosylation site (amino acids 53 to 55), included as a negative control for *in vitro* glycosylation, was not influenced by the presence of microsomal membranes (FIG.  
10 10A).

#### DISCUSSION

We have isolated and partially characterized a BDV glycoprotein with unusual properties. This protein,  
15 previously reported as 14.5 kDa {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}, is 16.2 kDa prior to carbohydrate modification and ~18 kDa after glycosylation. Though no classical sites for N linkage (N-x-S/T {Marshall, R. D., *Annu. Rev. Biochem.*, 41:673-702  
20 (1972)}) are found in the gp18 sequence, the protein is readily modified *in vitro* in the presence of a microsomal membrane system capable of N glycosylation {Gahmberg, C. G., et al., p. 281-297, *In* S. Fleischer and B. Fleischer (ed.), *Biomembranes*, Academic Press, New York (1983) and  
25 Lau, J. T. Y., et al., *J. Biol. Chem.*, 258:15255-15260 (1983)}. In addition, gp18 is sensitive to N-glycosidase F, an enzyme which cleaves between asparagine and N-acetylglucosamine {Plummer, T. H., et al., *J. Biol. Chem.*, 259:10700-10704 (1984) and Tarentino, A. L., et al.,  
30 *Biochem.*, 24:4665-4671 (1985)}. These findings indicate that gp18 is N glycosylated at a nonclassical site. One potential site is N-I-Y (amino acids 74 to 76). The presence of a hydroxyl amino acid (T or S) or cysteine in position +2 (N-x-T/S or C) has been proposed as essential  
35 for hydrogen bond donor function in N glycosylation

{Bause, E., et al., *Biochem. J.*, 195:639-644 (1981)}. It is possible that tyrosine (Y), another hydroxyl amino acid in position +2, could serve as a hydrogen bond donor in gp18. A second potential site for N glycosylation is L-  
5 N-S-L-S (amino acids 87 to 91), which is similar to S-N-S-G-phosphorylated S, the site for N glycosylation in a glycopeptide from hen yolk phosvitin {Shainkin, R., et al., *J. Biol. Chem.*, 246:2278-2284 (1971)}.

gp18 is sensitive to endoglycosidase F, an enzyme  
10 that cleaves after the N-linked N-acetylglucosamine in high mannose-, biantennary hybrid-, and biantennary complex-type oligosaccharides {Tarentino, A. L., et al., *Biochem.*, 24:4665-4671 (1985) and Tarentino, A. L., et al., *Methods Enzymol.*, 230:44-57 (1994)}. The protein is not  
15 sensitive to endoglycosidase H, an enzyme which cleaves after the N-linked N-acetylglucosamine in high-mannose- and most hybrid-type oligosaccharides but does not cleave complex-type oligosaccharides {Trimbel, R.B., et al., *Anal. Biochem.*, 141:515-522 (1984)}. Lectin staining using G.  
20 *nivalis* agglutinin shows no evidence of terminal mannose characteristic for hybrid- and high-mannose-type glycosylation. In contrast, staining with ConA (mannose, N-acetylglucosamine, branched trimannosyl core) {Ogata, S., et al., *J. Biochem.*, 78:687-696 (1975)}, wheat germ  
25 agglutinin (N-acetylglucosamine), and BS-II (terminal N-acetylglucosamine) {Ebisu, S., et al., *Methods Enzymol.*, 50:350-354 (1978)} indicates the presence of terminal N-acetylglucosamine and internal mannose. Thus, there is evidence from the pattern of endoglycosidase sensitivity  
30 and lectin staining that gp18 is likely to be a biantennary complex-type glycoprotein.

gp18 is sensitive to endo- $\beta$ -galactosidase. This enzyme cleaves between galactose and either N-acetylglucosamine or galactose when these saccharides  
35 occur in unbranched sequence {Scudder, P., et al., *J. Biol.*

*Chem.*, 259:6586-6592 (1984)}. The presence of galactose was confirmed by BS-I lectin binding (FIG. 8). The presence of both *N*-acetylglucosamine and galactose was confirmed by high-performance anion-exchange chromatography with pulsed amperometric detection. The combination of *N*-acetylgalactosamine and galactose is usually found in O-linked carbohydrates {Hayes, B. K., et al., *J. Biol. Chem.* 268:16170-16178 (1993)}. Though it is possible that gp18 is both N and O glycosylated, *N*-acetylgalactosamine has also been reported to occur in complex-type N-linked glycosylation {Hayes, B. K., et al., *J. Biol. Chem.* 268:16170-16178 (1993)}.

We did not detect a monocistronic ~429-nt mRNA for gp18 by PCR using oligo(dT), a 5' sense primer, and template from a variety of sources, including infected cell lines and rat brain. In contrast, a 429-nt gp18 cDNA was readily amplified by using gene-specific primers and total RNA or poly(A)<sup>+</sup> RNA as a template. Northern (RNA) hybridization experiments with gp18-specific probes using total RNA or poly(A)<sup>+</sup> RNA from infected cells or rat brain detected only 1.5-, 2.8-, 3.5-, 6.1-, and 7.1-kb transcripts. Recent experiments confirmed that the 1.5- and 2.8-kb RNAs can serve as templates for *in vitro* translation of the gp18 (data not shown). These data suggest that gp18 is likely to be translated from one or more of the larger RNA transcripts.

The role of gp18 in the BDV life cycle remains to be determined. Though the virus has not been characterized morphologically, genetic analysis has characterized BDV as a member of the order *Mononegavirales* {Briese, T., et al., *Proc. Natl. Acad. Sci. USA* 91:4362-4366 (1994) and Cubitt, B., et al., *J. Virol.*, 68:1382-1996 (1994)}. In nonsegmented, negative-strand RNA viruses, the third gene usually directs expression of a matrix protein. Matrix proteins in members of the order *Mononegavirales* are not known to be

glycosylated; however, glycosylated matrix proteins that resemble gp18 in size and pI (~10) have been found in other viral systems (e.g., E1 in coronaviruses {Armstrong, J., et al., *Nature* (London), 308:751-752 (1984)}).

5 Preliminary observations suggest that gp18 is present on the surface of the viral envelope. Monospecific antisera and monoclonal antibodies to gp18 precipitated viral particles and had neutralizing activity. In contrast, antibodies to p40 and p23 did not precipitate viral

10 particles or neutralize infectivity (see Example 4 below). Preincubation of primary rabbit fetal glia (cells highly susceptible to BDV) with gp18 prevented infection. No such effect was observed with either p40 or p23. Last, gp18 and BDV particles compete for binding

15 to a ~100-kDa membrane protein present in cells susceptible to infection.

#### Expression of Recombinant P57

20 cDNAs representing the p57 ORF were amplified by RT-PCR using BDV (strain He/80)-rat brain RNA as template. The amplified p57 cDNA was subcloned into two plasmid vectors, pET21b (Novagen) and pSFV-1 (GIBCO BRL).

pET21b, a prokaryotic expression vector, was selected because it allows for tight control of protein

25 expression, an important feature for expression of proteins toxic to host cells. The N-terminus of p57 contains a hydrophobic sequence that confers extreme toxicity to prokaryotic cells. Therefore, to facilitate the expression of p57, the first 152 N-terminal amino

30 acids were excluded during the cloning. PCR amplified cDNA representing nucleotides 2697 to 3743 of p57 ORF (amino acids 153 to 503) was generated by using oligonucleotide primers designed with a 5' restriction site (BamH1 for sense primer; Xho1 for antisense primer).

35 The PCR product was cloned into pET21b at the BamH1 and Xho1 restriction sites, thus generating pET21b-BDV57<sub>153-503</sub>. The pET21b-BDV57<sub>153-503</sub> plasmid was transformed into BL21

host cells and recombinant protein was expressed and purified by using protocols provided by the manufacturer.

An eukaryotic expression system, which allows for posttranslational modification, was selected for the expression of a recombinant protein more similar to native p57. pSFV-1 is a eukaryotic expression vector that can be used to generate a replication defective Semliki Forest virus (SFV) genomic RNA. The entire p57 ORF was PCR amplified and cloned into pSFV-1 prepared with 3' T-overhangs at the SmaI site, thus generating pSFV-BDV57. Transfection of pSFV-BDV57 transcripts into mammalian cells, results in overexpression of the posttranslationally processed p57 gene product.

15

### EXAMPLE 3

#### ELISA for the Detection of Antibodies to Borna Disease Virus Proteins

We have expressed p40, p23 and gpl8 as recombinant proteins and established a sensitive, specific ELISA for analyzing immunoreactivity to BDV. This assay system is more sensitive and rapid than methods currently employed for serologic diagnosis of infection such as Western blot, indirect immunofluorescent test (IFT) or immunoprecipitation.

This system provides a convenient tool for diagnosing disease, determining the prevalence of infection in animal and human populations and mapping the antigenic determinants for the immune response in infected hosts.

30

### **MATERIALS AND METHODS**

#### **Infection of Animals and Cultured Cells.**

Six week old Lewis rats (Charles River) were infected intranasally with  $6 \times 10^4$  focus forming units (ffu) of BDV strain He/80-1 {Carbone, K., et al., *J. Virol.*, 61:3431-3440 (1987) and Schneider, P. A., et al., *J. Virol.*, 68:63-68 (1994)}. C6 cells were persistently infected

with BDV He/80-1 (C6BDV) {Carbone, K. M., et al., *J. Virol.*, 67:1453-1460 (1993)}. Rabbit fetal glial cells were infected with BDV He/80-1 at a multiplicity of one ffu per cell then passaged once before use in IFT assays.

5 BDV strain He/80 was originally isolated from infected horse brain, passaged twice in rabbits, three times in rabbit fetal glial cells, and twice in Lewis rats {Herzog, S., et al., *Med. Microbiol. Immunol.*, 168:153-158 (1980)}. He/80-1 was passaged four additional times in

10 Lewis rats and used for infection of animals and cell lines.

**Generation of Recombinant Proteins (recp40, recp23, and recp18)**

15 Full length cDNAs encoding p40, p23 or gp18 were cloned into the prokaryotic expression vector pET15b (Novagen) for production of recombinant proteins. pBDV-40 in pcDNA II {McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992)} was amplified using the primers p4OXho I

20 (5'- CCCTCGAGGACCAAGATTT-3') (SEQ ID NO: 17) and Sp6 (20mer, Promega Corp., Madison, Wisconsin). pBDV-23 in pBluescript SKII+ {Thibault, K. J., M.S. thesis, University of California, Irvine (1992)} was amplified with the primers p24Nde I (5'- AGAATCATATGGCAACGCGACCATC-

25 3') (SEQ ID NO: 18) and T7 (20mer Promega). Polymerase chain reaction was performed using Taq polymerase (Perkin-Elmer Cetus Corp., Norfolk, Connecticut) according to the manufacturer's protocol. Products amplified from pBDV-40 and pBDV-23 were phenol/chloroform

30 extracted, precipitated and digested with BamH I and either Xho I (pBDV-40) or Nde I (pBDV-23) (Promega Corp., Madison, Wisconsin). pBDV-cpl8 in pBluescript SKII+ (see Example 2 above) was digested with Xho I and BamH I. Digested fragments were purified by agarose gel

35 electrophoresis (USB, USBioclean, Cleveland, Ohio) and cloned into pET15b (Novagen Corporation, Madison, Wisconsin). Protein expression in plasmid containing

*Escherichia coli* cells was induced by addition of isopropyl- $\beta$ -thiogalactopyranoside (1 mM) for 3 hours at 37°C. Proteins (recp40, recp23, and recp18) were purified by nickel-chelate affinity chromatography according to  
5 manufacturer's instructions (Novagen Corp.). Purification was assessed by SDS-PAGE and antigenicity was confirmed by Western blot using sera from infected rats. Proteins were dialyzed against 150 mM NaCl and 2.5 mM CaCl<sub>2</sub> and digested with biotinylated thrombin (1  
10 unit/mg recombinant protein, Novagen Corp.) overnight at room temperature. Thrombin was removed using streptavidin-agarose (Novagen Corp.) according to manufacturer's protocol. Protein concentrations were estimated by BioRad protein assay according to  
15 manufacturer's instructions.

#### **Antibodies to BDV and recombinant BDVproteins**

Sera were collected from infected rats at time of sacrifice or by tail bleeding at 2-week intervals after  
20 inoculation with BDV. Antibodies to recp40 and recp23 were each produced in two rabbits. Animals were injected subcutaneously (s.c.) with 25  $\mu$ g of protein in Freund's complete adjuvant and then boosted 3 weeks later s.c. with 25  $\mu$ g of protein in Freund's incomplete adjuvant.  
25 After 6 weeks some animals received an additional s.c. injection of 25  $\mu$ g protein in Freund's incomplete adjuvant. Blood was collected at 2-week intervals during weeks 7 through 14 for detection of antibodies by Western blot and ELISA.

30

#### **Indirect Immunofluorescent Test (IFT)**

Rabbit fetal glial cells were processed for titration of serum antibodies against BDV using the immunohistochemical methods of Pauli et al. {Pauli, G.,  
35 et al., *Zbl. Vet. Med. [B]* 31:552-557 (1984)}. Briefly, infected and noninfected cells were fixed with 4% formaldehyde in PBS, permeabilized with 1% Triton X-100

in PBS and blocked with 1% fetal bovine serum (FBS) in PBS. After incubation with sera diluted in 1% FBS in PBS, cells were incubated with fluorescein-conjugated goat anti-rat IgG and IgM or goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, Missouri) diluted 1:200 in 1% FBS in PBS and then examined by fluorescent microscopy. The IFT titer for each serum was determined to be the endpoint dilution at which specific immunofluorescence was detected.

10

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot (WB) and Immunoprecipitation (IP)**

For WB, lysates from infected and noninfected C6 cells were prepared according to Bause-Niedrig, et al. {Bause-Niedrig, I., M. et al., *Vet. Immunol. Immunopathol.*, 31:361-369 (1992)}. Proteins from these lysates (30 µg) and recombinant BDV proteins (250 ng) were subjected to 12% SDS-PAGE {Laemmli, U. K., et al., *J. Mol. Biol.*, 80:575-581 (1973)} and then transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, New Hampshire) {Towbin, H., et al., *Proc. Natl. Acad. Sci. USA*, 76:4350-4354 (1979)}. Membranes were incubated at room temperature first with WB-diluent (0.5% nonfat dry milk (Carnation Company, Los Angeles, California) and 0.05% Tween-20 (Fisher Scientific, Raleigh, North Carolina) in TBS (tris balanced saline, 50 mM Tris-HCl pH 7.5 and 150 mM NaCl)) for one hour, then overnight with various dilutions (1:10 to 1:2,000) of rat sera or monospecific rabbit sera in WB-diluent. Membranes were washed 3 times in TBS, incubated for 2 hours with the appropriate secondary antibody (horseradish peroxidase-conjugated goat anti-rat IgG and IgM or goat anti-rabbit IgG, Sigma Chemical Co., St. Louis, Missouri) diluted 1:500 in WB-diluent, washed 5 times in TBS and then incubated with hydrogen peroxide and 4-chloro-1-naphthol (Pierce Chemical Company, Rockford, Illinois) according to manufacturer's



instructions. Methods for synthesis and analysis of radiolabeled BDV proteins and immunoprecipitation have been described {Lipkin, W. I., et al., *Proc. Natl. Acad. Sci., USA*, 87:4184-4188 (1990)}. Briefly, plasmid clones pBDV-gp18, pBDV-23 and pBDV-40 were linearized and used as template for *in vitro* transcription and translation of [<sup>35</sup>S] methionine-labeled proteins. After precipitation with rat or rabbit sera and protein A-sepharose (Sigma Chemical Co., St. Louis, Missouri), proteins were analyzed by SDS-PAGE and autoradiography.

#### ELISA

Ninety-six well, Immulon I microtiter plates with lids (Dynatech Laboratories, Chantilly, Virginia) were coated overnight at 37°C with 10 ng of recombinant protein per well in 100 µl of borate buffer (100 mM boric acid, 50 mM sodium borate and 75 mM sodium chloride, pH 8.4). Plates were washed three times with washing buffer (0.05% Tween-20 in PBS) and incubated for 1 hour at 37°C with ELISA-diluent (0.5% bovine serum albumin (BSA) fraction V (USB) in washing buffer). Two-fold serial dilutions of sera were prepared in ELISA-diluent; 100 µl of sera diluted from 1:250 to 1:500,000 was then added to each well and incubated for 2 hours at 37°C. Plates were washed three times with washing buffer. Next, 100 µl of horseradish peroxidase-conjugated goat anti-rat IgG and IgM (Sigma Chemical Co., St. Louis, Missouri) diluted 1:5,000 in ELISA-diluent were added to each well and incubated for 1 hour at 37°C. After washing the plates five times, 100 µl of substrate solution was added to each well. Substrate solution consisted of 9.9 ml of 100 mM sodium acetate adjusted to pH 6.0 with 100 mM citric acid, 100 µl of 10 mg of 3,3',5,5'-tetramethylbenzidine (Sigma Chemical Co., St. Louis, Missouri) per ml in dimethyl sulfoxide and 1.5 µl of 30% hydrogen peroxide (Fisher Scientific, Raleigh, North Carolina). After incubation in the dark at room temperature for 30

minutes, the reaction was stopped by the addition of 50  $\mu$ l of 25% sulphuric acid (Sigma Chemical Co., St. Louis, Missouri) to each well. The absorbance at 450 nm was determined for each well using a microplate reader (Molecular Devices, Thermo max, Menlo Park, California). Negative control wells, without primary antisera, were used for calibration. The ELISA titer for each serum was defined as the endpoint dilution that yielded an optical density of 0.3.

10

## RESULTS

The figures below present some of the results:

FIG. 11. Western blot analysis of native and recombinant proteins with monospecific antisera to recombinant proteins and sera from infected rats. Recombinant viral proteins and lysates from infected C6BDV or noninfected C6BDV cells were size-fractionated and screened by Western blot. A) Sera from infected and noninfected rats were used to detect native or recombinant proteins. Lane 1, C6BDV lysate; lane 2, recp40; lane 3, recp23; lane 4, recp18; lane 5, C6 lysate; lane 6, recp40, recp23 and recp18. Lanes 1-4 were treated with serum from infected rat; lanes 5 and 6 were treated with serum from noninfected rat. B) Monospecific antisera were used to detect BDV-specific proteins. C6BDV lysates (lanes 1-3) and C6 lysates (lanes 4 and 5) were incubated with: lanes 1 and 4, serum from infected rat; lane 2, anti-p40 rabbit serum; lane 3, anti-p23 rabbit serum; and lane 5, pooled anti-p40 and anti-p23 sera.

FIG. 12. ELISA of infected rat serum reacted with recp40. ELISA was performed with 10 ng/well recp40 or BSA as described in Materials and Methods. Circles, recp40 and serum from chronically infected rat; squares, recp40 and serum from noninfected rat; triangles, BSA and serum from chronically infected rat.

FIG. 13. Timecourse for appearance of antibodies to BDV-proteins. Sera were collected at different times post-infection and assayed by ELISA for antibodies to (A) recp40; (B) recp23; and (C) recp18. Error bars represent standard error of the mean. Number of animals analyzed at each time point: <4 wks, 15; 5 wks, 6; 6 wks, 12; 8 wks, 4; 10 wks, 5; and 15 wks, 9.

#### Production of recombinant viral proteins and monospecific antisera to recombinant viral proteins

Full length coding sequences for p40, p23 and gp18 were expressed in *Escherichia coli* and recombinant proteins were purified. The yield of protein in 100 ml of bacterial culture was: recp40, 1 mg; recp23, 500 µg; and recp18, 50 µg. Recombinant proteins were analyzed by SDS-PAGE. A predominant band of the expected molecular weight was observed for each protein and tested for antigenicity by WB using sera from BDV-infected and noninfected rats (FIG. 11A). Recombinant proteins were detected by sera from BDV-infected rats but not by sera from noninfected rats. Recombinant proteins, recp40 and recp23 were used to produce antibodies in rabbits. The production of antibodies was monitored by ELISA. Rabbits were sacrificed when the ELISA titer reached 1:500,000 (week 16 of immunization). The specificity of the antisera was then tested by WB using lysates from infected cells and recombinant proteins (FIG. 11B). Antisera were monospecific: rabbits immunized with recp40 produced antibodies that reacted only with p40 and recp40; rabbits immunized with recp23 produced antibodies that reacted only with p23 and recp23. At week 16 of immunization, the antisera were also titered by IFT. Antisera to recp40 and recp23 had IFT titers of 1:50,000 and 1:100,000, respectively.

### **Specificity and sensitivity demonstrated in the BDV-ELISA systems**

In order to establish a sensitive and specific ELISA for all three recombinant BDV proteins, the optimal antigen concentration was determined by checkerboard titration of positive and negative sera versus various antigen concentrations. For each protein, the concentration that resulted in the most linear response was 10 ng/well. The sensitivity of the ELISA system for each recombinant protein was established using sera from infected rats known to be reactive by IFT, IP and WB. For each of the proteins, 100% of sera that had been found to be positive by other methods were also positive by ELISA. Specificity was tested using sera from noninfected rats. ELISA for each protein proved to be highly specific for detection of antibodies to BDV proteins: recp40-ELISA with noninfected rat sera showed 80% specificity at 1:500 dilution or 100% specificity at 1:2,000, recp23-ELISA showed 93% specificity at 1:250 and 100% specificity at 1:1,000, recp18-ELISA showed 100% specificity at 1:250. Figure 12 shows a representative ELISA using recp40 as target antigen. Various dilutions of sera from chronically infected and noninfected rats were tested with 10 ng of recombinant protein or BSA per well in comparison with BSA. No nonspecific background reactivity was observed at serum dilutions of 1:500 or higher (FIG. 12). Results were similar when recp23 and recp18 were used as target antigen.

### **Analysis of immunoreactivity to viral proteins by IFT WB, IP and ELISA in sera from infected rats.**

Adult rats infected intranasally with BDV did not display abnormal behaviors prior to the fourth week post-infection (predisease, PD). Four to six weeks post-infection, in the acute phase of disease (AD), animals had hyperactivity, weight loss, disheveled fur, dystonic posture and hindlimb paresis. Eight to fifteen weeks

post-infection, signs of disease stabilized: there was no additional weight loss, hyperactivity diminished and paresis did not progress. This chronic phase of the disease (CD) persisted for the life of the animals. Sera  
5 was collected from adult-infected rats between 3 and 15 weeks after infection with BDV, and analyzed for the presence of antibodies to viral proteins using four different methods: IFT, WB, IP and ELISA (Table 3).

TABLE 3 Detection of BDV-specific antibodies in sera from infected rats by different methods

Serum	WB				IP <sup>a</sup>			Reciprocal ELISA titer <sup>b</sup>			Reciprocal IFT titer
	recp40	recp23	recp18		p40	p23	p18	recp40	recp23	recp18	
PD (3-4 wk pi <sup>c</sup> ; n = 15)	-	-	-	-	-	-	-	2,388 ± 256	904 ± 181	163 ± 5 <sup>d</sup>	<10
AD (4-6 wk pi; n = 18)	+	+	-	-	+	+	-	3,217 ± 829	2,644 ± 20	279 ± 19	20-200
CD (10-15 wk pi; n = 14)	+	+	+	+	+	+	+	291,889 ± 56,590	76,527 ± 19,309	4,680 ± 1,467	10,000-20,000

<sup>a</sup> In vitro-translated proteins.<sup>b</sup> Values are mean ± standard error of the mean titer.<sup>c</sup> pi, postinfection.<sup>d</sup> Nonspecific. Value below the level of specificity of the recp18 ELISA (1:250).

IFT allowed detection of antibodies to BDV in both AD rats and CD rats. In AD rats, the titer was between 1:20 and 1:200, whereas in CD rats, the titer was between 1:10,000 and 1:20,000. Sera from PD rats were not reactive by IFT. WB using lysates from infected cells or recombinant proteins, and IP using proteins translated *in vitro* yielded identical results: sera from CD animals were reactive with p40, p23 and gp18; sera from AD rats detected only p40 and p23; sera from PD rats did not react with p40, p23 or gp18. ELISA detected antibodies reactive with p40, p23 and gp18 in sera from all CD and AD rats (Table 3). In PD rats, ELISA only detected antibodies reactive with p40 and p23; immunoreactivity with gp18 was below specificity (Table 3).

The timecourse for the appearance of antibodies to BDV-proteins in sera was determined by ELISA. Sera collected at regular intervals from adult-infected rats were tested in the recp40, recp23 and recp18 ELISA systems. Titers of antibodies to all three proteins increased throughout the period of observation from weeks 4 to 15 post infection (FIG. 13).

## DISCUSSION

Three recombinant BDV proteins, recp40, recp23 and recp18, were expressed and used as immunogens for production of monospecific sera in rabbits. Two of these antisera, directed against recp40 and recp23, are reported here; antisera to recp18 are described in Example 4 below. These three recombinant proteins were detected by sera from infected rats (FIG. 11A) and by monoclonal antibodies to purified native proteins. Monospecific antisera to the recombinant proteins were immunogen-specific as determined by WB (FIG. 11B) and detected proteins in infected cells by IFT.

ELISA systems were established, based on recombinant proteins, that have several advantages over methods currently used for detection of BDV-specific antibodies

including IFT, WB and IP. Although IFT is widely accepted as a method for diagnosing BDV infection and titering antibodies to the virus, it has two disadvantages. First, IFT does not define the viral protein(s) responsible for immunoreactivity. Second, as shown here, IFT titers are 10-100 fold less sensitive than ELISA for detection of antibodies to p40 or p23. This relative insensitivity resulted in failure of IFT to show evidence of infection in PD rats (Table 3). WB and IP allowed detection of antibodies to individual viral proteins but were also less sensitive than ELISA. Sera from PD rats were not reactive by either WB or IP.

For diagnostic purposes, the recp40-ELISA is the most sensitive method for detection of antibodies in infected animals. Antibodies to recp40 were present prior to disease onset and had higher titers than antibodies to recp23 or recp18. Although the recp23-ELISA was also positive in PD and AD rats, the recp18-ELISA was not. Because high titer antibodies to gp18 only appear in chronic disease, the recp18-ELISA may be used to estimate the duration of infection. Low antibody titers to recp18 are not due to the lack of glycosylation on this recombinant protein because similar ELISA titers were found with native gp18 antigen. Failure to produce high titer antibody response to recp18 may be due to lower levels of expression of this protein than p40 or p23.

Growing recognition that BDV has a broader species and geographic range than previously appreciated suggests the importance of designing sensitive, reliable assays for infection. The ELISA systems described here, provide inexpensive, rapid methods for BDV-serology. In contrast to IFT, WB and IP, which require at least 2 days for completion and are not well suited to screening multiple samples, ELISA allows analysis of hundreds of sera in several hours with only minimal equipment. Plates coated with these proteins have been stable in ELISA for up to



one month at room temperature and thus are practical for use in remote laboratories. In addition to serving as a tool for clinical diagnosis and epidemiology of Borna disease infection, the BDV ELISA is a useful tool for studies in immunopathogenesis and virus biology. For example, applicants have mapped antigen binding sites on p40 and p23 by ELISA using sera from infected animals and monoclonal antibodies to BDV proteins.

Dependent on the population studied and the methods used for analysis (WB, IP or IFT), the prevalence of antibodies reactive with BDV proteins in patients with neuropsychiatric disorders has been estimated to be between 4% and 23% {Bode, L., In W. I. Lipkin and H. Koprowski (ed.), Borna Disease. Springer-Verlag, Heidelberg, in press (1995)}. Variability between laboratories could be due to differences in populations analyzed, antigen preparations or experimental technique. The BDV ELISA based on recombinant proteins provides a standardized method for investigating human immunoreactivity to this neurotropic infectious agent.

#### EXAMPLE 4

##### Neutralizing Antibodies in BDV Infected Animals

We examined the timecourse for the development of neutralization activity and the expression of antibodies to individual BDV viral proteins in sera of infected rats. The appearance of neutralizing activity correlated with the development of immunoreactivity to gp18, but not p40 or p23. Monospecific and monoclonal antibodies to native gp18 and recombinant non-glycosylated gp18 were also found to have neutralizing activity and to immunoprecipitate viral particles or subparticles. These findings suggest that gp18 is likely to be present on the surface of the viral particles and to contain epitopes important for virus neutralization.

Antibodies to p40 and p23 (soluble antigens) are readily detected in both sera and cerebrospinal fluid

(CSF) of naturally and experimentally infected animals {Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988); Ludwig, H., et al., *Arch. Virol.*, 55:209-223 (1977) and Ludwig, H., et al., *Med. Microbiol. Immunol.*, 163:215-226  
5 (1977)}. Antibodies to gp18, a membrane-associated glycoprotein (previously described as 14.5 kDa), have been reported less frequently {Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988) and Rubin, S. A., et al., *J. Virol.*, 67:548-52 (1993)}. Although neutralization  
10 activity has been found in sera of animals infected with BDV {Danner, K., et al., *Zbl. Vet.-Med. [B]*, 25:345-355 (1978); Hirano, N., et al., *J. Gen. Virol.*, 64:1521-1530 (1983); Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988) and Ludwig, H., et al., *Arch. Virol. [Suppl]* 7:111-133 (1993)}, the  
15 antibodies responsible for neutralization activity have not been investigated. An enzyme-linked immunosorbent assay (ELISA) based on recombinant BDV proteins has been established in Example 3 above, that provides a sensitive method for detection of antibodies to gp18. We find that  
20 the appearance of neutralizing antibodies in infected rats correlates with immunological reactivity to gp18. Furthermore, monospecific and monoclonal antibodies (MAbs) directed against gp18 neutralize BDV infectivity and immunoprecipitate viral particles or subparticles.

25

#### MATERIALS AND METHODS

**BDV infected animals:** Sixty-thousand focus forming units (ffu) of BDV strain He/80-1 {Carbone, K. M., et al., *J. Virol.*, 61:3431-3440 (1987); Herzog, S., et al., *Med. Microbiol. Immunol.*, 168:158-8 (1980) and Schneider, P. A., et al., *J. Virol.*, 68:63-68 (1994)} were used to intranasally  
30 (i.n.) infect each of seventy 6-week old Lewis rats. Rats were observed at three days intervals for weight loss, ruffled fur or postural abnormalities consistent  
35 with acute disease. Sera were collected at time of

sacrifice. Under metofane anesthesia, rats were perfused with buffered 4% paraformaldehyde; brains were fixed overnight in perfusate at 4°C. Twenty-micron sagittal sections were collected onto gelatin coated slides and stained with hematoxylin and eosin. Inflammation was scored using the scale of Stitz, Sobbe and Bilzer {Stitz, L., et al., *J. Virol.*, 66:3316-23 (1992)}.

#### Virus titration and neutralization assay.

10        Viral infectivity in 20% brain homogenates was determined using the method of Pauli et al. {Pauli, G., et al., *Zbl. Vet.-Med. [B]* 31:552-557 (1984)}. Virus neutralization was performed using a modification of Danner et al. {Danner, K., et al., *Zbl. Vet.-Med. [B]*, 25:345-15 355 (1978)}. Briefly, 50 ffu of BDV were incubated with serial dilutions of antibodies or sera for one hour at 37°C, added to rabbit fetal glial cells and incubated for 5 days. Sera was heat inactivated at 56°C for 30 minutes. In selected assays, mouse complement (1:50) 20 (Sigma Chemical Co., St. Louis, Missouri) was added to the virus concurrent with the addition of MAbs to determine the effects of complement on neutralization activity. The dilution of serum or antibody required to reduce the number of ffu by 50% was defined as the 25 neutralization titer (NT<sub>50</sub>). As controls for each neutralization assay, rabbit fetal cells were exposed to medium without virus, treated with virus in medium alone (no antibodies), or treated with virus incubated with sera from normal rats. Pilot studies showed that 30 approximately 8% of normal rat sera interfered with BDV infectivity at dilutions up to 1:16. Therefore, sera were considered to be neutralizing only if the NT<sub>50</sub> exceeded 1:32. Supernatant from nonproducing myeloma cell lines as well as monoclonal antibodies directed 35 against BDV-p23 (24/36F1) and BDV-p40 (38/17C1) {Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)} were found to neutralize infectivity at dilutions of 1:2.

Thus, monoclonal antibodies were considered to be neutralizing only if the NT50 exceeded 1:4.

**Preparation of proteins (recp40, recp23, recp18 and gp18):**

Plasmids encoding p40 {pBDV-40 disclosed in McClure, M. A., et al., *J. Virol.*, 66:6572-6577 (1992)}, p23 {pBDV-23 disclosed in Thibault, K. J., M.S. thesis; University of California, Irvine (1992)} and gp18 {pBDV-gp18 disclosed in Kliche, S. et al., *J. Virol.*, 68:6918-6923 and Example 2 above} were subcloned (see Example 3 above) into the prokaryotic expression vector pet15b (Novagen, Madison, Wisconsin). Recombinant proteins (recp40, recp23 and recp18) were expressed in *Escherichia coli* and purified according to manufacturer's protocol (Novagen, Madison, Wisconsin). Purity and antigenicity were assessed by SDS-PAGE and Western blot analysis using sera from infected rats. Native, glycosylated gp18 was prepared from infected rat brain as described previously {Schädler, R., et al., *J. Gen. Virol.*, 66:2479-2484 (1985)}.

**Enzyme-linked immunosorbent assay (ELISA):**

ELISA was performed as described in Example 3 above. Briefly, plates coated with recombinant protein were incubated with serially diluted sera or MAbs. Bound horseradish peroxidase (HRPO)-coupled secondary antibody (goat anti-mouse F'ab-HRPO, goat anti-rat IgG and IgM HRPO; Sigma Chemical Co.) was quantified on a microplate reader (Thermo max, Molecular Devices, Menlo Park, California) using the chromagen 3,3'-5,5' Tetramethylbenzidine (Sigma Chemical Co.). The ELISA endpoint titer was defined as the serum or antibody dilution that generated an optical density of 0.3.

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot and Immunoprecipitation (IP):**

Recombinant or native BDV proteins were subjected to SDS-PAGE {Laemmli, U. K., et al., *J. Mol. Biol.*, 80:575-581  
5 (1973)} and transferred to nitrocellulose (Schleicher & Schuell, Inc., Keene, New Hampshire) or Immobilon-N membranes (Millipore Corp., Bedford, Maryland) {Towbin, H., et al., *Proc. Natl. Acad. Sci. USA*, 76:4350-4354 (1979)}. Membranes were blocked and incubated with primary  
10 antibody as described in Example 3 above. After incubation with secondary antibody (goat antimouse IgG-alkaline phosphatase [AP], goat anti-rat IgG-AP or goat anti-rat IgG and IgM-HRPO, Sigma Chemical Co., St. Louis, Missouri), immune complexes were visualized using Western  
15 Blue (Promega, Madison, Wisconsin) for AP or chemiluminescence (ECL kit, Amersham, Arlington Heights, Illinois) for HRPO according to manufacturer's instructions. gp18 or recp18 were precipitated using sera from infected rats, monospecific antibodies or MAbs  
20 and Protein A-Sepharose (Pharmacia Biotech Inc., Piscataway, New Jersey) as described by Persson, H., et al. {Persson, H., et al. *Science*, 225:687-693 (1984)} then assayed by Western blot.

**25 Monoclonal antibodies:**

MAbs to gp18 were generated according to Thiedemann et al. {Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)}. Briefly, Balb/c mice were immunized intraperitoneally (i.p.) with 5  $\mu$ g of gp18 in complete  
30 Freund's adjuvant. Three and 6 weeks after the initial immunization, mice were boosted i.p. with 5  $\mu$ g of gp18 in incomplete Freund's adjuvant. Four days before fusion of spleen cells with the mouse myeloma cells X63-Ag8.653 {Kearney, J. F., et al., *J. Immunol.*, 123:1548-1550 (1979)},  
35 mice were boosted intravenously with 15  $\mu$ g of gp18. All hybridomas were initially screened for reactivity to gp18

by ELISA. Tissue culture supernatants from positive hybridomas were concentrated by ammonium sulfate precipitation {Jonak, Z. L., p. 405-406, *In* R. H. Kennett, T. J. McKean, and K. B. Bechtol (ed.), "Monoclonal antibodies, Hybridomas: A new dimension in biological analyses", Plenum Press, New York (1982)} and tested by Western blot and IP for reactivity with gp18 and recp18. The immunoglobulin isotype was determined using an agglutination isotyping kit (Serotec, Oxford, England) according to manufacturer's instructions. Monoclonal antibody, 24/36F1 directed against BDV-p23 {Thiedemann, N., et al., *J. Gen. Virol.*, 73:1057-1064 (1992)}, was used as a negative control in Western blot and IP experiments.

**15 Generation of polyclonal sera against recp18 protein:**

To produce antibodies against recp18, two 2-month old Lewis rats were injected subcutaneously (s.c.) with 25 µg of protein in Freund's complete adjuvant and boosted 3 weeks later with 25 µg of protein s.c. in Freund's incomplete adjuvant. After 6 weeks, animals received i.p. injections of 25 µg protein in phosphate buffered saline (PBS) with 20 µg lipopolysaccharide (*S. typhimurium*, Difco, Detroit, Michigan) at two-week intervals for a total of three injections. Serum was collected every two weeks during weeks 7 through 14 for analysis by ELISA and Western blot and for determination of neutralization titer. Mouse antibodies to native gp18 have been described in Example 2 above.

**30 Affinity adsorption of BDV-specific serum-antibodies:**

Antibodies that bound to recp23 and recp40 were sequentially removed from serum of an infected rat according to Crabb et. al. {Crabb, B. S., et al., *Virology*, 190:143-154 (1992)}. Serum (D2) from an adult-infected Lewis rat (15 weeks post intranasal infection), was diluted 1:10 in TBS (tris balanced saline, 50 mM Tris pH

7.4 and 100 mM NaCl) and incubated overnight at 4°C with membrane-bound recp23. The anti-recp23 antibody-depleted serum (D2 Δαrecp23) was removed, the membrane was washed with TBS and adsorbed anti-recp23 antibodies were eluted  
5 (recp23 eluant) by incubation with 1 ml of 0.1 M glycine, 0.15 M NaCl pH 2.7 for 3 minutes. The pH of the eluant was adjusted by addition of 300 μl of 10 mM Tris HCl pH 7.5. The anti-recp23 antibody-depleted serum was then incubated with membrane-bound recp40 (D2 Δαrecp23,  
10 Δαrecp40) and purified as before (recp40 eluant). Antibody depletion from serum and antibody elution from membrane-bound proteins was monitored by Western blot and ELISA. At each step during the purification, antibody-depleted sera and eluted antibodies were analyzed for  
15 neutralizing activity. Antibodies to gp18 or recp18 were also adsorbed (D2 Δαgp18, D2 Δαrecp18) and eluted (gp18 eluant, recp18 eluant) by this method. These adsorption and elution experiments were repeated using serum (B3) from an additional adult-infected rat (15 week post  
20 intranasal infection).

**IP of BDV particles or sub particles and analysis by reverse transcription polymerase chain reaction (PCR):**

Forty-thousand ffu of BDV in a volume of 200 μl  
25 were treated with 50 μg/ml of DNase I and RNase A (Boehringer Mannheim Corp., Indianapolis, Indiana) for 30 minutes at 37°C then incubated for 2 hours at room temperature with 100 μl of one of the following: (1) serum from acutely or chronically infected rats at 1:10  
30 dilution in PBS; (2) purified serum-antibodies at 1:10 dilution; (3) mouse anti-gp18 sera or rat anti-recp18 sera at 1:20 dilution; or (4) monoclonal antibodies against gp18 at 1:5 dilution. Next, 100 μl of 1 mg/ml Protein A-Sepharose (Pharmacia, Piscataway, New Jersey)  
35 in PBS was added, and the mixture was incubated overnight at 4°C. The Protein A-Sepharose-antibody-virus complex was washed three times in PBS then resuspended in 100 μl

water. Total RNA was extracted {Chomczynski, P., et al., *Anal. Biochem.*, 162: 156-159 (1987)} and used for RT-PCR amplification of a 693 nucleotide region of the viral genome (nucleotide 753 to 1446) according to Schneider et al. (primer 7 and primer 9) {Schneider, P. A., et al., *J. Virol.*, 68:63-68 (1994)}. PCR products were analyzed by agarose gel electrophoresis. PCR products were cloned and sequenced to confirm that they represented the predicted region of the genomic RNA {Schneider, P. A., et al., *J. Virol.*, 68:63-68 (1994)}. Negative controls for RT-PCR included the omission of virus from immunoprecipitation reactions and the use of genomic sense primers during first strand cDNA synthesis.

## RESULTS

15

The following figures present part of the results:

FIG. 14. Timecourse for the appearance of antibodies to BDV proteins in sera from individual rats after i.n. infection. (A) Neutralization activity in sera from BDV-infected rats at three timepoints (5, 10 and 15 weeks post-infection). Each serum is represented by a circle. Bars indicate mean neutralization titer for each group (5, 10 or 15 weeks post-infection). Asterisk represents sera with neutralization titer less than or equal to 1:16. (B) Plot of mean recp18 ELISA titers (open columns) with neutralization titers (hatched columns) at three time points (5, 10 and 15 weeks post-infection). Sera analyzed were the same as those in panel A. Mean values for neutralization activity were determined as described in FIG. 14A. Arrows indicate threshold for significance in neutralization assay (1:32) and recp18 ELISA (1:250). These values were selected because normal rat sera reacted in the neutralization assay and recp18 ELISA at titers of 1:16 and 1:125, respectively. (C) Timecourse for the appearance of antibodies to recp40, recp23, and gp18 by Western blot analysis. Proteins were size-fractionated by SDS-PAGE



and transferred to nitrocellulose membranes. Membranes were incubated first with sera and then with horseradish peroxidase-coupled goat anti-rat IgG. Bound secondary antibody was detected by chemiluminescence. Results shown are from serum of one representative animal at several different timepoints post BDV infection (p.i.).

FIG. 15. Monoclonal antibody (MAb) detection of gp18. A) Immunoprecipitation of gp18 with MABs. gp18 was first incubated with MABs or sera from infected or noninfected rats, then precipitated with Protein-A Sepharose, size-fractionated by 12% SDS-PAGE and transferred to Immobilon-N membranes. Precipitated gp18 was visualized with rat anti-recp18 sera, goat anti-rat IgG-AP, and Western Blue. Lanes 1, serum from infected rat (15 week p.i.); 2, serum from noninfected rat; 3, MAB 14/29A5; 4, MAB 14/26B9; 5, MAB 14/8E1; 6, MAB 14/13E10; 7, MAB 14/18H7; 8, MAB 24/36F1 (MAB directed against the BDV 23 kDa protein, negative control); 9, no antibody. Arrow indicates gp18; H and L represent heavy and light chains of immunoglobulin, respectively. B) MABs were analyzed for binding to native gp18 in Western blot. gp18 was separated on 12% SDS-PAGE and transferred to an Immobilon-N membrane. Strips were incubated with MABs or sera from infected or noninfected rats. Bound antibodies were detected with alkaline phosphatase conjugated goat anti-rat IgG or goat anti-mouse Fab-specific and Western Blue substrate. Lanes: 1, serum from infected rat (15 week p.i., D2); 2, serum from noninfected rat; 3, MAB 14/29A5; 4, MAB 14/26B9; 5, MAB 14/8E1; 6, MAB 14/13E10; 7, MAB 14/18H7; and 8, MAB 24/36F1 (MAB directed against the BDV 23 kDa protein, negative control). Molecular weight markers ( $10^3$  Da) are shown at the right.

FIG. 16. Neutralization profile of sera and MABs. BDV (50 ffu) was preincubated with serial dilutions of serum or MAB and then added to ten thousand rabbit fetal glial cells. After four days of incubation, the infected cells were visualized as described in Pauli et al.

{Pauli, G., et al., *Zbl. Vet.-Med. [B]* 31:552-557 (1984)}. The number of infected cell-foci per well was counted. (A) Serum from noninfected rat. (B) serum from infected rat (15 week p.i., D2). (C) MAb 14/13E10. (D) MAb 14/29A5.

5        FIG. 17. Precipitation of BDV using sera from infected rats, monospecific rat antisera to recp18 and monoclonal antibodies (MAbs) to gp18. Virus was treated with nucleases to eliminate nucleic acid not contained within virions then immunoprecipitated with sera or MAbs  
10      and Protein A-Sepharose. RNA was extracted and subjected to RT-PCR to amplify a 693 nucleotide viral genomic sequence. PCR-products were visualized in an ethidium bromide-stained 1% agarose gel. (A) Precipitation of BDV with sera from infected rats. Lanes: 1, serum from  
15      infected rat, 15 week p.i.; 2, serum from infected rat, 5 week p.i.; 3, serum from infected rat, 15 week p.i., no BDV; 4, serum from infected rat, 15 week p.i., genome sense primer used for first strand cDNA synthesis. (B)  
20      Precipitation of BDV by monospecific antisera to recp18 and MAbs to gp18. Lanes: 1, monospecific rat antisera to recp18; 2, MAb 14/13E10; 3, MAb 14/29A5. DNA markers (basepairs) are shown at the right.

**Timecourse of disease and appearance of antibodies to BDV  
25      in infected rates:**

Rats developed Borna disease (BD) within 5 weeks post infection. The acute phase of the disease, 4-8 weeks post infection, was associated with marked weight loss, disheveled fur, dystonic posture, hind limb paresis  
30      and paralysis, mortality of 35%, and prominent inflammatory cell infiltrates in the brain. In the chronic phase of disease, 10-15 weeks post-infection, signs of disease stabilized and inflammation receded. Virus titers in the brains of animals acutely (5 weeks  
35      p.i.) and chronically infected (15 weeks p.i.) were  $2.4 \pm 0.4 \times 10^5$  ffu/ml and  $4.4 \pm 0.2 \times 10^4$  ffu/ml, respectively.

Sera were monitored for virus neutralization activity (FIG. 14A, B and C) and the presence of antibodies reactive with recp40, recp23, recp18 or native gp18 in Western blot (FIG. 14C) and ELISA.

5 Neutralization activity was first detected in sera (28% of the animals) at 5 weeks p.i. By week 15 p.i., all sera had neutralization activity with a mean titer of 1:977 $\pm$ 246. Antibodies to recp18 were first detected by ELISA at week 5 p.i. and showed a marked increase in

10 titer by 15 weeks p.i. (1:4,610 $\pm$ 1,463) (FIG. 14B). In contrast, antibodies reactive with recp40 and recp23 were detected by ELISA within 4 weeks of infection, reached a titer greater than 1:20,000 by 8 weeks p.i. and remained elevated through 15 weeks p.i. (see Example 3 above).

15 Antibodies reactive with recp40 and recp23 were detected by Western blot between weeks four and five p.i., whereas antibodies to gp18 were detectable only after week 10 p.i. (FIG. 14C).

20 **Affinity adsorption of neutralizing sera:**

To determine whether the presence of antibodies to gp18 correlate with neutralization activity, two rat sera (D2 and B3, 15 weeks p.i.), were tested in the neutralization assay after successive depletions of

25 antibodies to individual BDV-proteins. Antibodies to BDV-specific proteins were removed from D2 rat serum by adsorption with membrane-bound protein. The efficiency of antibody depletion from serum was monitored by Western blot and ELISA. Prior to adsorption, the titers to

30 recp40 and recp23 were each greater than 1:20,000. Following adsorption with recp23, the titer to recp23 decreased to 1:200. After adsorption with recp40, the titer to recp40 decreased to 1:150. Eluted antibodies were reactive by ELISA with the proteins used for

35 adsorption: recp23 eluant titer, 1:5,000; recp40 eluant titer, 1:15,000. Serum antibodies remaining after adsorption, and eluted antibodies, were then tested for

neutralizing activity. The neutralization titer of the D2 serum ( $NT_{50}$  1:1,000-1,500) did not change after adsorption with recp23 and recp40 antigens (D2  $\Delta\alpha$ recp23,  $\Delta\alpha$ recp40) (Table 4). Antibodies eluted from proteins  
5 recp40 (recp40 eluant) and recp23 (recp23 eluant) had no neutralization activity (Table 4). In contrast, the  $NT_{50}$  of the D2 serum decreased from 1:1,000-1,500 to 1:600-700 after adsorption with recp18 (D2  $\Delta\alpha$ recp18) and to 1:160-200 after adsorption with gp18 (D2  $\Delta\alpha$ gp18) (Table 5).  
10 The neutralization titers of antibodies eluted from recp18 (recp18 eluant) and gp18 (gp18 eluant) were 1:60-100 and 1:240-400, respectively (Table 4). Similar results were obtained with serum from rat B3.

TABLE 4 Characterization of serum antibodies

Serum	Reciprocal NT	IP-RT-PCR <sup>a</sup>	Reciprocal gp18 ELISA titer
Chronic (15 wk p.i. [D2])	1,000–1,500	+	4,300–5,000
D2 Δarecp23, Δarecp40 <sup>b</sup>	1,000–1,500	+	4,000–5,000
D2 Δarecp18 <sup>b</sup>	600–700	+	NS <sup>c</sup>
D2 Δagp18 <sup>b</sup>	160–200	+	800–840
recp18 eluant <sup>d</sup>	60–100	+	2,400–3,000
gp18 eluant <sup>d</sup>	240–400	+	1,200–2,000
recp23 eluant <sup>d</sup>	NS	–	NS
recp40 eluant <sup>d</sup>	NS	–	NS
Ratarecp18	320–480	+	>5,000
Mouseagp18	160–320	+	>5,000

<sup>a</sup> IP of BDV and detection of genomic RNA by RT-PCR.

<sup>b</sup> Chronic rat sera (D2) adsorbed with recombinant (recp23, recp40, recp18) or native (gp18) protein.

<sup>c</sup> NS, not significant. The NT was considered to be not significant below 1:32; the recp18 ELISA titer was considered to be not significant below 1:250.

<sup>d</sup> Antibodies in chronic rat sera (D2) eluted from recombinant or native protein.

TABLE 5 Characterization of anti-gp18 MAbs

MAb	Ig class	Reciprocal NT	Characterization by:				Reciprocal rp18 ELISA titer	IP-RT-PCR*
			Western blot		IP			
			gp18	rp18	gp18	rp18		
14/29A5	IgG2b	>400-1,000	+	+	+	+	500-1,000	-
14/26B9	IgM	8-16	-	-	+	+	4-8	+
14/8E1	IgM	50	-	-	+	+	200-300	+
14/13E10	IgM	50-100	-	-	+	+	16-64	+
14/18H7	IgG3	100-200	-	-	+	+	128-256	+

\* IP of BDV and detection of genomic RNA by RT-PCR.

**Monospecific antibodies to recp18 and gp18:**

Sera from rats and mice immunized with recp18 and gp18, respectively, were tested for neutralization activity. Neutralizing antibodies in both rats and mice  
5 were detected at 12 weeks post-immunization. Sixteen-weeks after immunization with recp18, 2 rats had neutralization titers between 1:320 and 1:480 (Table 4); sera from 2 mice immunized with gp18 had neutralization titers between 1:160 and 1:320 (Table 4).

10

**Monoclonal antibodies to gp18:**

MABs were generated against gp18. Five positive clones were identified by ELISA using gp18 as antigen. The MABs represented three different immunoglobulin  
15 isotypes, yet all contained the kappa light chain (Table 5). Although each of the monoclonal antibodies immunoprecipitated gp18 (Table 5, FIG. 5A) and recp18 (Table 5), only MAB, 14/29A5 reacted by Western blot (Table 5, FIG. 15B).

20 Concentrated supernatants from all five MABs neutralized BDV infectivity (Table 5). Similar to sera from chronically-infected rats (FIG. 16B), the neutralization titer of four MABs was greatest at highest antibody concentration (FIG. 16C). In contrast, one MAB,  
25 14/29A5, neutralized BDV only when used at a dilution of 1:400-1:1,000 (FIG. 16D). Neutralizing sera from chronically-infected rats, mice immunized with gp18 or rats immunized with recp18 (Table 4) had the capacity to inhibit 100% of BDV infectivity (FIG. 16B). In contrast,  
30 MABs to gp18, used individually or in concert, inhibited a maximum of 68% of BDV infectivity (FIG. 16C and D). Supernatants of two MABS, 14/18H7, NT<sub>50</sub> 1:16 and 14/13ElO, NT<sub>50</sub> 1:32, showed cooperativity in neutralization assays; pooling of these MABs resulted in a higher neutralization  
35 titer (NT<sub>50</sub> 1:100-150). To determine the extent to which neutralization was complement-dependent, neutralization activity of MABs was tested with addition of either

active or heat-inactivated mouse complement. No increase in neutralization titer was detected with addition of mouse complement. Serum from noninfected (normal) rats was not neutralizing at dilutions greater than 1:16 (FIG. 5 16A).

#### Immunoprecipitation of BDV with neutralizing antibodies:

BDV stock was treated with nucleases to eliminate free nucleic acids then incubated with sera or MABs and Protein A-Sepharose. RNA was extracted from immunoprecipitated viral particles or subparticles and subjected to RT-PCR for amplification of viral genomic RNA. Neutralizing rat sera (FIG. 17A), monospecific sera to recp18 (FIG. 17B) or gp18, and D2 serum antibodies 15 eluted from recp18 or gp18 precipitated BDV particles. Removal of antibodies to recp23, recp40, recp18 or gp18 did not affect the capacity of neutralizing sera to precipitate viral particles. Four MABs also precipitated BDV (FIG. 17B and Table 5). One MAB, 14/29A5, did not 20 precipitate viral particles at any dilution (1:5, 1:100, 1:200 or 1:500). Sera from noninfected or two acutely infected rats (5 weeks p.i.) (FIG. 17A) did not precipitate BDV. Experiments with sera and monoclonal antibodies are summarized in Tables 4 and 5. Negative 25 controls for RT-PCR included the omission of virus from immunoprecipitation (FIG. 17A) and the use of genomic sense primers for first strand cDNA synthesis (FIG. 17A).

#### DISCUSSION

30 The presence (or absence) of neutralizing antibodies in BDV-infected animals has been controversial. Some reports have not shown evidence for neutralizing antibodies {Carbone, K. M., et al., *J. Virol.*, 61:3431-3440 (1987); Herzog, S., et al., *J. Gen. Virol.*, 66:503-8 (1985) and Narayan, O., et al., *J. Inf. Dis.*, 148:305-315 (1983)}, 35 however, this may reflect different timepoints for collection of sera or variation in the assay system for



neutralization. Although there are reports of neutralizing antibodies in serum and CSF of both naturally and experimentally infected animals {Danner, K., et al., *Zbl. Vet.-Med. [B]*, 25:345-355 (1978); Hirano, N., et al., *J. Gen. Virol.*, 64:1521-1530 (1983); Ludwig, H., et al., *Progr. Med. Virol.*, 35:107-151 (1988) and Ludwig, H., et al., *Arch. Virol. [Suppl]* 7:111-133 (1993)}, neither the timecourse for development of neutralizing antibodies nor their target antigens have been characterized. Here, we show that the neutralizing activity of BDV-rat sera increases dramatically from the acute (5 weeks p.i.) to the chronic (15 weeks p.i.) phase of disease and provide evidence to indicate that neutralization activity is due, at least in part, to antibodies that react with a BDV glycoprotein, gp18. The timecourse for the appearance of neutralizing antibodies seems to correlate with immunoreactivity to gp18. Furthermore, removal of antibodies to gp18 or recp18 dramatically decreased the neutralization titer of BDV-rat sera. In contrast, subtraction of antibodies to two other viral proteins, p40 and p23, had no effect.

Neutralization activity was detected with monospecific antiserum against both gp18 and recp18 as well as with monoclonal antibodies against gp18. These MAb represent three different isotypes, IgM, IgG2b and IgG3, indicating that multiple isotypes are capable of virus neutralization. Addition of complement did not enhance neutralization activity of the MAbs, suggesting that the mechanism for neutralization was neither complement-mediated inactivation of virus nor steric hindrance by a complement-MAb-virus complex.

It is likely that at least three different antibody binding sites on gp18 were involved in neutralization. Four MAbs, which immunoprecipitated both gp18 and recp18 but did not detect protein in Western blots, presumably bound to discontinuous epitopes. The observation that use of MAbs 14/13E10 and 14/18H7 in combination, resulted

in greater neutralization activity than use of either MAb alone, suggests that these MAbs recognized either different discontinuous epitopes or different binding sites on a single discontinuous epitope. One MAb, 14/29A5, detected protein in Western blots as well as immunoprecipitation assays indicating that it bound to a continuous epitope. Unlike the other MAbs, 14/29A5 neutralized infectivity only after dilution (FIG. 16D), a profile consistent with neutralization by virus aggregation as reported in other viral systems {Dimmock, N. J., A. Capron, et al. (ed.), "Current Topics in Microbiology and Immunology", Springer-Verlag, Berlin (1993) and Outlaw, M. C., et al., *J. Gen. Virol.*, 71:69-76 (1990)}. Although all of the gp18 MAbs detected recp18 (nonglycosylated protein), it is possible that there are additional epitopes for neutralization which include the carbohydrate portion of gp18.

Sera from chronically-infected rats had greater neutralization activity than monospecific sera or monoclonal antibodies directed against gp18. Higher neutralization activity in sera from infected animals could reflect factors that influence epitope presentation such as interactions between gp18 and other proteins or the virion envelope. Alternatively, gp18 may not be the only BDV protein that elicits neutralizing antibodies. Sera from chronically-infected animals retained partial neutralizing activity and the capacity to precipitate virus after adsorption with gp18. Although this may be due to incomplete subtraction of antibodies to gp18 (Table 4) neutralizing antibodies may be directed against other viral proteins as well. For example, an additional candidate for a virion surface protein that may elicit neutralizing antibodies is p57. This putative protein contains multiple potential N-glycosylation sites and, as the product of the fourth ORF on the BDV genome, is in the gene position generally occupied by glycoproteins in *Mononegavirales* {Briese, T., et al., *Proc. Natl. Acad. Sci. USA*:

91:4362-4366 (1994)}. It is contemplated that passive administration of neutralizing antibodies or immunization with gp18 and other virion surface proteins can alter BDV pathogenesis.

5

#### EXAMPLE 5

##### Fragments of Borna Disease Virus Proteins Immunoactive With Sera From Human Schizophrenics and BDV Infected Animals

10       The etiology of schizophrenia, a debilitating disease that affects approximately 1% of the world's population, is unknown. Higher prevalence in some geographic areas, seasonal variation in births of subjects who develop disease, increased risk of  
15 schizophrenia in subjects exposed to influenza virus during the second trimester *in utero* and discordance for disease in monozygotic twins suggest the possibility of an infectious basis {Kirch, D. G., *Schizophrenia Bulletin*, 19:355-370 (1993)}. Borna disease virus has been  
20 implicated in human affective disorders by studies reporting that patients have serum antibodies to BDV {Rott, R., *et al.*, *Science*, 228:755-756 (1985)} and the presence of viral proteins and nucleic acids in peripheral blood mononuclear cells {Bode, L., *et al.*, *Nature*  
25 *Medicine*, 1:232-236 (1995)}. The catecholamine-related stereotypic behaviors observed in BDV-infected rats and catecholamine system dysfunction present in schizophrenia prompted Western blot (WB) studies of sera from schizophrenics for antibodies to BDV obtained from  
30 animals infected with the virus {Waltrip, R. W., II, *et al.*, *Psychiatry Res.*, 56:33-44 (1995)}. The present invention discloses an ELISA test for schizophrenia and BDV infection; fragments and peptides derived from p23 and gp18 which are immunoreactive with sera from  
35 schizophrenics and animals infected with BDV and/or immunized with p23 and gp18. The test is specific,

sensitive, fast, easy, and economical. For example, indirect immunofluorescence assays (IFT) used in the current art does not define the viral protein(s) responsible for immunoreactivity. IFT, immunoprecipitation (IP), and WB are also less sensitive than the ELISA of the present invention and require at least 2 days for completion and are unsuitable for screening of multiple samples. In contrast, the present ELISA provides inexpensive, rapid tests which allow analysis of hundreds of serum samples, e.g. in several hours, with minimal equipment. The advantages of ELISA over the prior art diagnostic methods are described in further detail in Briese, T., *et al.*, *J. Clin. Microbiol.*, 33:348-351 (1995). Besides the detection of schizophrenia and BDV infections disclosed in this Example, the ELISA method disclosed herein is generally applicable for studies and detection of neurologic and neuropsychiatric diseases and BDV infections in men and animals.

20 I. Immunoreactivity of p40, p23 and gp18, with Sera from Schizophrenics.

Sera from 30 human schizophrenic patients were examined by ELISA for immunoreactivity with recombinant BDV proteins N (recp40), P (recp23) and M (recp18) {The recombinant proteins were produced and the ELISA were performed as described in Examples 3 and 4, above, which were also described Briese, T., *et al.*, *J. Clin. Microbiol.*, 33:348-351 (1995)}. Controls were sera from 30 age and sex matched normal subjects and 30 patients with multiple sclerosis (MS), an autoimmune central nervous system (CNS) disease of unknown etiology. Although some sera detected N or P but not M, all sera immunoreactive with M also detected N and P. Twenty-seven percent of schizophrenic subjects (7) had serum antibodies to M, N and P versus 3% of normal subjects (1) or 0% of MS patients ( $p < 0.0001$ ) (Table 6). Immunoreactivity of sera with all 3 proteins was confirmed by WB using extracts

from BDV-infected C6 cells supplemented with gp18; IP of the recombinant BDV proteins and IFT using infected rabbit fetal glial cells (the WB, IFT, and IP were conducted using the methods known in the art, as  
5 described in Briese, T., *et al.*, *J. Clin. Microbiol.*, 33:348-351 (1995)). Sera not reactive with 1 or more of the 3 proteins in ELISA were also negative in the other assays.

TABLE 6

Percentage of subjects with schizophrenia (SZ), multiple sclerosis (MS) or no neuropsychiatric disease (NND) immunoreactive in ELISA with N, P and M proteins of

Borna disease virus							
	N*	P†	M‡	N/P†	N/M‡	M/P†	N/P/M‡
SZ	32	47	27	33	27	27	27
(n=30)							
MS	0	0	0	0	0	0	0
(n=30)							
NND	7	7	3	7	3	3	3
(n=30)							

N = p40, P = p23, M = gp18

\* p < 0.01; † p < 0.001; ‡ p < 0.0001

N/P = N and P

N/M = N and M

M/P = M and P

N/P/M = N, P and M

## II. Selection and Immunoreactivity of Truncated Fragments of p23 and gp18

The immunologic determinants on p23 and gp18 were determined using truncated fragments of these proteins.

5 The fragments used are shown in Figs. 20B and 21B. In FIG. 20B, the fragments are designated S1 to S4 and NS, respectively. In FIG. 21B, the fragments are derived from the unglycosylated version of recp18, and are denoted M1 to M4 and MS. The fragments are shown from  
10 the amino terminus (left) to the carboxyl terminus (right) of the proteins. The numbers below each fragment indicate the locations of the amino acids on the full length p23 or gp18, respectively. For example, p23 has a total of 201 amino acids, thus, as shown in FIG. 20B,  
15 S1 represents the full length p23 because it spans from amino acid at position 1 (denoted 1aa in the figure) to the amino acid at position 201 (denoted 201aa) of p23. S2 is a protein representing a fragment of p23, spanning from amino acid at position 37 to position 201 of p23.  
20 Similarly, in FIG. 21B, MS is a protein representing a fragment of the unglycosylated gp18, spanning from amino acid at position 1 to position 70 of the unglycosylated gp18. These fragments were recombinantly produced by selecting the appropriate PCR primers for the fragments  
25 based on the cDNA of p23 and gp18 disclosed in this patent application and by cloning the respective cDNA fragments into the prokaryotic expression vector pET15b (Novagen) using techniques known in the art such as described in Example 3, above.

30 To test their immunoreactivity, these truncated fragments of p23 and gp18 proteins were used in ELISA with sera from 6 BDV infected rats (15 weeks post infection) and 7 immunoreactive schizophrenic patients (of Section I above). Horse sera were only from acutely  
35 infected animals, at a stage of disease where antibodies to gp18 are not present, thus, sera from 4 BDV infected horses were used only to study p23. For truncated

fragments of p23, similar patterns of immunoreactivity were found for sera from BDV infected rats, BDV infected horses and schizophrenic patients. In the case of truncated fragments of unglycosylated gp18 proteins, instead of using sera from BDV infected horses, the sera from 2 mice immunized with native gp18 were tested (15 weeks post immunization). The immunized mice were used to determine whether the truncated fragments derived from unglycosylated gp18 were specifically immunoreactive with antibodies raised against native gp18. Again, similar patterns of immunoreactivity were found for sera from the BDV infected rats, mice immunized with native gp18, and schizophrenic patients. The above results are shown in Fig 20A and 21A, respectively, the taller blocks in the histograms indicate increased immunoreactivity relative to the shorter blocks. Significantly, the above truncated fragments did not immunoreact with sera from the same controls (and the same number of controls) used in Section I above, whereas some sera from human controls with no neuropsychiatric disease immunoreacted with the full length p23 and gp18 proteins (see Table 6). Thus, these fragments are more specific for detecting neuropsychiatric disease than the full length proteins.

### 25 III. Epitope Mapping of Peptides Derived from p23 and gp18

Fine-mapping of epitopes with overlapping peptides of p23 and gp18 also revealed that the same determinants were detected by the above sera. To determine where an epitope was within each protein, a series of overlapping peptides were chemically synthesized and each peptide was tested for its ability to bind the antibody from schizophrenics and the sera of animals infected with BDV and/or immunized with p23 and gp18.

35 As shown in Figs. 22 and 23, peptides of 8-mers were chemically synthesized, starting from the amino terminus of p23 and gp18 and spanning the full length of the



proteins. Except for the peptides at the amino and carboxyl termini of p23 and gp18, each of the intervening peptide overlaps its neighboring peptides (at its amino and carboxyl ends, respectively) by 4 amino acids.

- 5 To map the immunoepitopes on p23, the above 8-mer peptides derived from recp23 were tested against sera from: 6 rats infected with BDV (15 weeks post infection, p.I.); 2 rabbits immunized with recp23 (15 weeks post immunization); and 7 immunoreactive schizophrenic
- 10 patients (of Section I above). To map the immunoepitopes on gp18, the above 8-mer peptides derived from unglycosylated recp18 were similarly tested, except that the rabbit sera were replaced with sera from 2 mice immunized with native gp18 (15 weeks post immunization).
- 15 The immunized mice were used to determine whether the series of overlapping 8-mer peptides derived from unglycosylated gp18 were specifically immunoreactive with antibodies raised against native gp18. The controls in both tests were the same as in Section I above.
- 20 The tests were conducted using SPOTs membrane (Genosys Biotechnologies, Inc., The Woodlands, TX, USA) and the technique described in Frank, R., *et al.*, *Tetrahedron*, 44:6031-6040 (1988); Blankenmeyer-Menge, B., *et al.*, *Tetrahedron Letters*, 29(46):5871-5874 (1988); Blankenmeyer-
- 25 Menge, B., *et al.*, in "Innovation and Perspectives in Solid-Phase Synthesis", (Epton, R. ed.), Chapman and Hall publ. (1989); and Blankenmeyer-Menge, B., *et al.*, *Tetrahedron Letters*, 32(12):1701-1704 (1990) (the tests are hereinafter described as the "SPOTs tests"). The results are shown
- 30 in Figs. 22 and 23. The blocks in the histogram in each figure indicate the peptides which immunoreact with the sera, the taller block indicates increased immunoreactivity relative to the shorter blocks. Based on the immunoreactivity, the sequences of the epitopes
- 35 were deduced. The amino acid sequences of the epitopes and thus the peptides are as shown in Tables 7 and 8,

below, wherein the peptide sequences are shown from the amino terminus (left) to the carboxyl terminus (right):

**TABLE 7**

5	Peptides derived from p23	
	MATRPSSL	SEQ ID No. 20
	NALTQPV DQLLK	SEQ ID No. 21
	DQPTGREQ	SEQ ID No. 22
	VRGTLGDI	SEQ ID No. 23
10	TAQRCDHS	SEQ ID No. 24
	METMKLMM EKVD	SEQ ID No. 25
	PMLPSHPA	SEQ ID No. 26
	TADEWDII	SEQ ID No. 27

**TABLE 8**

15	Peptides derived from gp18	
	MNSKHSYV	SEQ ID No. 28
20	TLMLEIDF	SEQ ID No. 29
	GHSLVNIYFQID	SEQ ID No. 30
	YKDPIRKY	SEQ ID No. 31
	AFNVFSYR	SEQ ID No. 32

The result of representative SPOTs tests with the 8-mer peptides derived from unglycosylated gp18 are graphically shown in FIG. 24A, the panels contained sera from: 1 mouse immunized with native gp18, 1 rat infected with BDV, and 1 schizophrenic human, respectively. Each spot on the panels indicates the immunoreaction of a serum sample with an 8-mer unglycosylated gp18 peptide. As shown in the scale on Fig 24B, the darker the spots, the higher the immunoreactivity. The lightest spot (Scale 1) indicates no detectable immunoreactivity; and the darkest spot (Scale 4) indicates highest immunoreactivity. As shown in FIG. 24A, the immunoreactivity pattern of the sera against the peptides were similar for all the animals/humans tested. Based on the pattern of

immunoreactivity as shown by the spots, the epitopes E1 to E5 were mapped. The result of the epitope mapping is graphically shown in FIG. 24B, the height of the blocks is directly proportional to the degree of immunoreactivity of the peptides tested which span the full length of gp18, from amino acid at position 1 to position 142. The sequences of the mapped epitopes, E1 to E5, are listed below the histogram of FIG. 24B. The epitopes mapped are the same as in Table 8, above, and confirmed that the sera were specifically immunoreactive with epitopes found within gp18. Again, significantly, the control sera did not immunoreact with the peptides.

The same test was applied to the overlapping 8-mer peptides derived from p23, except that the mice were immunized with recp23. A similar result was obtained, *i.e.* the immunoreactivity pattern of the sera against the peptides were similar for all the animals/humans tested, and the test produced the epitopes shown in Table 7, above. Again, significantly, the control sera did not immunoreact with the peptides.

In summary, the above truncated fragments, epitopes and peptides, and nucleotide sequences which encode them or which are complementary to these encoding nucleotide sequences, can be used to: (1) diagnose, prognose, monitor, and manage BDV infection/disease and schizophrenia, and more generally neurologic and neuropsychiatric diseases; and (2) vaccinate an animal or human against the foregoing infection and diseases. Other useful truncated immunoreactive fragments, epitopes and peptides can be similarly derived from the other BDV proteins using the method of this Example. Thus, the nucleotide sequences encoding these truncated fragments, epitopes and peptides, nucleotide sequences complementary to the foregoing, and recombinant vectors and cells expressing the truncated fragments, epitopes and peptides, and their uses are also claimed here. The vaccines, diagnostic, prognostic and monitoring methods,

recombinant vectors and cells, and nucleotide sequences, can be made using the teaching contained in this patent application in combination with methods known in the art. The above findings also suggest an association between  
5 BDV infection and schizophrenia.

#### EXAMPLE 6

##### Identification and Characterization of the BDV G-Protein

10 Although the BDV antigenome contains five major ORFs, products are reported only for the first three ORFs on the antigenome: N (p40), P (p24/p23) and M (gp18). The fourth ORF predicts a protein (G-protein) of 57 kDa that contains potential N-glycosylation sites. We have  
15 used a Semliki forest virus (SFV) vector to express the fourth ORF in BHK-21 cultured cells. The expressed protein migrated at 94 kDa in a 10% SDS-PAGE analysis. A 94 kDa BDV-specific protein was also identified in infected C6 cells by immunoprecipitation with sera from  
20 infected rats. The expressed protein was markedly sensitive to tunicamycin, endoglycosidase F/N-glycosidase and endoglycosidase H, indicating that the protein is an N-linked glycoprotein, largely comprised of high mannose- and/or hybrid-type oligosaccharides.  
25 SFVp57 transfected cells showed surface expression of the protein and formed syncytia. The protein's presence on the surface of transfected cells supports the hypothesis that the G-protein may be a virion surface attachment protein.

30 The fourth ORF in BDV predicts a protein of 57 kDa with N- and O- glycosylation sites and hydrophobic domains. Our findings show that in fact the fourth ORF encodes a BDV G-protein of approximately 94 kDa with multiple N-glycosylation and O-glycosylation sites and  
35 hydrophobic domains at the amino and carboxyl termini reminiscent of the signal sequence and transmembrane domains found in rhabdovirus G-proteins (FIG. 25). FIG.

25 shows the predicted amino acid sequence of the BDV G-protein (this BDV G-protein is also referred to as p57 in this patent application, and the amino acid sequence is listed as SEQ ID No. 8, above). Boxed regions  
5 represent the putative endoplasmic reticulum ("ER") signal peptide sequence (amino acids 7 to 20) and transmembrane domain (amino acids 468 to 488). Bold underlined sequences represent potential N-glycosylation sites. We now report that this ORF directs the  
10 expression of a 94-kDa N-linked glycoprotein.

**Expression of a 94 kDa protein from the BDV p57 ORF using an SFV expression vector.** The p57 ORF (nt. 2229 to nt. 3744; Strain V) {Briese, T., *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:4362-4366 (1994)} was amplified by RT-PCR from  
15 BDV-infected C6 cell RNA by using primers 5'-CGCAATCAATGCAGC (SEQ ID NO 34) and 5'-TTCCTGCCACCGGCCG (SEQ ID NO 35) and cloned into the SmaI restriction site in vector pSFV-1 (GibcoBRL, Life Technologies, Inc.,  
20 Grand Island, New York) {Marchuk, D., *et al.*, *Nucleic Acids Res.*, 19:1154 (1990)} to create pSFVp57. Capped SFV genomic RNA encoding p57 or  $\beta$ -galactosidase (control), were transcribed from pSFVp57 or pSFV3LacZ template, respectively. After tranfection into BHK-21 cells, the  
25 over expressed proteins were analyzed by immunohistochemical and biochemical assays {Kriegler, M., *In Gene transfer and expression: a laboratory manual*, p. 219-224, Stockton Press, New York (1990)}. The SFVp57 cells formed large multinucleated syncytia and expressed a  
30 surface protein detected by sera from BDV infected rats ("BD-rat sera" or "BDSe") but not normal rat sera (NLSe). SFVLacZ cells did not form syncytia, or express proteins reactive with either BDSe or NLSe. The syncytia formation and surface protein expression observed in  
35 cells transfected with SFV-p57 were similar to those described in cells transfected with SFV vectors containing other glycoproteins {Gallagher, W. R., *et al.*, *J.*

*Virol.*, 14:813-820 (1974); Paul, N. L., *et al.*, *AIDS Res. and Hum. Retroviruses*, 9:963-970 (1993)}.

Lysates of metabolically-labeled SFVp57 and SFVLacZ cells were used for immunoprecipitation (IP) experiments with BDSe and NLSe. Approximately  $2 \times 10^4$  transfected cells (16 hrs after electroporation) were incubated for two hours in 1 ml of methionine-minus Modified Eagles Medium (GibcoBRL, Life Technologies, Inc., Grand Island, New York). Thereafter, 0.2mCi of  $^{35}\text{S}$ Met-Cys-protein label mix (New England Nuclear, Boston, Massachusetts) was added for eight hours to radiolabel newly synthesized proteins. Cell lysates were subjected to IP according to Yamashita *et al.* {Yamashita, Y., *et al.*, *J. Virol.*, 68:7933-7943 (1994)} except for the modification that protein G-sepharose (Sigma) was substituted for protein A. After SDS-PAGE and autoradiography, a 94 kDa protein was detected in lysates of SFVp57 cells but not in lysates of SFVLacZ cells.

**Identification of a BDV-specific 94 kDa protein in BDV-infected C6 cells.** Lysates of BDV-infected C6 cells (C6BDV) and non-infected C6 cells were metabolically-labeled and IP with BDSe or NLSe for analysis by SDS-PAGE and autoradiography (see above). At least six BDV-specific proteins were detected in lysates from infected cells by BDSe that were not detected in lysates of infected cells by NLSe or in lysates of noninfected cells by BDSe. These included proteins of 200 kDa (pol), 94 kDa (G-protein), 40 kDa (N protein), 36 kDa, 33 kDa and 23 kDa (P protein). Whether the 36 kDa or 33 kDa proteins are of viral or host origin is unknown.

**Characterization of the BDV 94 kDa protein.** Metabolically-labeled SFV-p57 and SFVLacZ cells were treated with 10  $\mu\text{g/ml}$  tunicamycin to inhibit N-linked glycosylation. Cell lysates were used in IP experiments with BDSe or NLSe prior to SDS-PAGE and autoradiography.

BDS<sub>e</sub> immunoprecipitated proteins of 94 kDa and 64 kDa from SFVp57 cell lysates. The 94 kDa protein was detected in untreated cells but not in tunicamycin-treated cells. Conversely, the 64 kDa protein was  
5 detected in treated cells but not in untreated cells. Neither protein was detected in SFVLacZ cells.

Radiolabeled proteins immunoprecipitated by BDS<sub>e</sub> were eluted from the sepharose beads by incubation in sixty microliters of 50 mM Tris-HCl, pH 6.8, 0.4% SDS,  
10 0.1M 2-mercaptoethanol at 95°C for 10 min and digested with endoglycosidase H (Endo H) (Boehringer Mannheim); endoglycosidase F and N-glycosidase F (Endo F/PNGase F) (J. Elder); Endo H, neuraminidase (Boehringer Mannheim) and O-glycosidase (Boehringer Mannheim); or neuraminidase  
15 and O-glycosidase. Methods for carbohydrate digestion followed protocols of the manufacturer (Endo H, neuraminidase, O-glycosidase) or Alexander and Elder (Endo F/PNGase F) {Alexander, S., *et al.*, *Meth. Enzymol.*, 179:505-518 (1989)}; using Endo H, 2.0 mU; Endo F/PNGase  
20 F, 25 mU; O-glycosidase, 0.8 mU; neuraminidase, 1.0 mU in 40 microliter reactions. Controls for these reactions included incubation of proteins with digestion buffer alone. Radiolabeled p57 was prepared by *in vitro* translation in rabbit reticulocytes in the absence of  
25 microsomal membranes to provide a nonglycosylated G-protein standard {Lipkin, W. I., *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:4184-4188 (1990)}. Proteins were subjected to 10% SDS-PAGE and analyzed by autoradiography. Treatment with endo H or endo F/PNGase F resulted in an apparent  
30 shift in approximately mw from 94 kDa to 64 kDa, the position of radiolabeled nonglycosylated G-protein. Treatment with neuraminidase and O-glycosidase did not allow resolution of a shift. Therefore, to enhance the sensitivity of SDS-PAGE for identifying small shifts in  
35 mw following O-glycosidase digestion, protein was first incubated with endo H. Comparison of protein digested with endo H alone with protein digested with endo H,

neuraminidase and O-glycosidase revealed a subtle shift of less than 1 kDa.

Antibodies to the BDV 94 kDa protein in BDV-infected  
5 rats. Viral glycoproteins tend to be immunoreactive and  
are often targets for neutralizing antibodies. Previous  
studies of the BDV M-protein revealed epitopes that bind  
neutralizing antibodies {Hatalski, C. G., *et al.*, *J. Virol.*,  
69:741-747 (1995)}. Because adsorption experiments using  
10 purified M-protein did not completely abrogate  
neutralization activity in chronic BDSe, it was proposed  
that additional neutralization epitopes might be present  
on the putative G-protein {Hatalski, C. G., *et al.*, *J. Virol.*,  
69:741-747 (1995)}. To address the possibility that  
15 serum antibodies to G-protein might be present at higher  
titer in chronic BD-rat, sera from BD-rats sacrificed at  
different phases of disease were used to IP proteins from  
metabolically-labeled SFVp57 cells for analysis by SDS-  
PAGE and autoradiography. The 94 kDa protein signal was  
20 approximately 10-fold higher after IP with sera collected  
from animals 3 months post infection (chronic phase) than  
after IP with sera collected from animals 1 month post  
infection (acute phase).

This study was initiated to identify and  
25 characterize the product of the fourth ORF on the BDV  
antigenome. Expression of this ORF in SFVp57 cells  
yielded a 94 kDa glycoprotein. Inhibition of  
carbohydrate conjugation by tunicamycin and endo F/PNGase  
F sensitivity indicate a primary role for N-linkage.  
30 Sensitivity to endo H suggests that the N-linked  
carbohydrate is largely comprised of high mannose- and/or  
hybrid-type oligosaccharides {Tarentino, A. L., *et al.*,  
*Methods Enzymol.*, 50:574-580 (1979)}. The subtle shift in  
apparent mw after digestion with neuraminidase and O-  
35 glycosidase may represent the presence of O-linked



carbohydrate {Hawkins, L. K., *et al.*, *Viol.*, 210:335-344 (1995)}.

Although there are no direct data to indicate a function for BDV G-protein, several observations suggest  
5 that it could play a role in viral attachment and/or penetration. First, the BDV G-protein contains a carboxyl transmembrane domain and localizes to the plasma membrane in pSFV57 cells. These features are reminiscent of other enveloped viral systems where G-  
10 proteins mediate early events in infection {White, J., *et al.*, *Q. Rev. Biol. Phys.*, 16:151-195 (1983)}. Second, treatment of BDV particle preparations with N-acetylglucosaminidase and mannosidase reduced infectivity suggesting the importance of N-acetylglucosamine and mannose residues in  
15 terminal positions {Stoyloff, R., *et al.*, *Arch. Virol.*, 137:405-409 (1994)}. Although the BDV M-protein may serve as a viral attachment protein, it does not contain terminal mannose residues {Kliche, S., *et al.*, *J. Virol.*, 68:6918-6923 (1994)}. In contrast, the BDV G-protein  
20 does include terminal mannose residues and could therefore represent the sensitive virion surface component identified in particle infectivity experiments. Third, the titer of antibodies to G-protein in infected rats increased dramatically in the chronic phase of  
25 infection, consistent with the timecourse for appearance of neutralizing antibodies {Hatalski, C. G., *et al.*, *J. Virol.*, 69:741-747 (1995)}.

All publications and patent applications mentioned  
30 in this Specification are herein incorporated by reference to the same extent as if each of them had been individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for  
35 purposes of clarity and understanding, it will be obvious that various modifications and changes which are within

the skill of those skilled in the art are considered to fall within the scope of the appended claims. Future technological advancements which allows for obvious changes in the basic invention herein are also within the  
5 claims.

#### Deposit

The cDNA of BDV genomic RNA sequence has been deposited in the GenBank data base (accession no.  
10 U04608). This GenBank sequence is hereby incorporated by reference in its entirety.

The recombinant transfer vector, suitable for transformation into *Escherichia coli* DH10, containing four overlapping cDNA libraries (as described in Example 1,  
15 above) representing the entire BDV viral genome has been deposited under the Budapest Treaty, at the American Type Culture Collection, Rockville, MD 20852 (U.S.A.) on December 30, 1994 under the deposit name BDVU04608, and ATCC Accession No. 97008.

20 Availability of the deposited recombinant transfer vector is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

25 Also, the present invention is not to be considered limited in scope by the deposited recombinant transfer vector, since the deposited vector is intended only to be illustrative of particular aspects of the invention. Any recombinant transfer vector which can be used to prepare  
30 recombinant microorganism which can function to produce a recombinant protein product described herein is considered to be within the scope of this invention. Further, various modifications of the invention in addition to those shown and described herein which are  
35 apparent to those skilled in the art from the preceding description are considered to fall within the scope of the appended claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: The Regents of the University of California
- (ii) TITLE OF INVENTION: Borne Disease Viral Sequences,  
Diagnostics and Therapeutics for Central Nervous  
System Diseases
- (iii) NUMBER OF SEQUENCES: 35
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Robbins, Berliner & Carson
  - (B) STREET: 201 N. Figueroa St., Suite 500
  - (C) CITY: Los Angeles
  - (D) STATE: California
  - (E) COUNTRY: USA
  - (F) ZIP: 90012-2628
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/369,822
  - APPLICATION NUMBER: US 08/434,831
  - APPLICATION NUMBER: Unknown
  - (B) FILING DATE: 06-JAN-1995
  - FILING DATE: 04-MAY-1995
  - FILING DATE: 04-JANUARY-1996
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Berliner, Robert
  - (B) REGISTRATION NUMBER: 20,121
  - (C) REFERENCE/DOCKET NUMBER: 5555-357C2
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 213/977-1001
  - (B) TELEFAX: 213/977-1003

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1112 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGCCACCCA AGAGACGCCT GGTGATGAC GCCGATGCCA TGGAGGATCA AGATCTATAT	60
GAACCCCCAG CGAGCCTCCC TAAGCTCCCT GGGAAATTC TACAATACAC CGTTGGGGGG	120
TCTGACCCGC ATCCGGGTAT AGGGCATGAG AAAGACATCA GGCAGAACGC AGTGGCATTG	180
TTAGACCAGT CACGGCGCGA TATGTTTCAC ACAGTAACGC CTAGCCTTGT GTTCTATGT	240

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TTGCTAATCC CAGGACTGCA CGGTGCGTTT GTTCACGGAG GGGTGCCTCG TGAATCCTAC 300  
 CTGTCGACGC CTGTCACCGC TGGAGAACAG ACTGTTGTTA AGACTGCGAA GTTTTACGGG 360  
 GAAAAGACGA CGCAGCGTGA TCTCACCAG CTGGAGATCT CCTCTATCTT CAGCCATTGT 420  
 TGCTCATTAC TAATAGGGGT TGTGATAGGA TCGTCGTCTA AGATCAAAGC AGGAGCCGAG 480  
 CAGATCAAGA AAAGGTTTAA AACTATGATG GCAGCCTTAA ACCGGCCATC CCATGGTGAG 540  
 ACTGCTACAC TACTCCAGAT GTTTAATCCA CATGAGGCTA TAGATTGGAT TAACGGCCAA 600  
 CCCTGGGTAG GTCCTTTGT GTTGTCTCTA CTAACACAG ACTTTGAGTC CCCAGGTAAA 660  
 GAATTTATGG ACCAGATTAA GCTTGTGCGA AGTTATGCAC AGATGACTAC GTACACTACT 720  
 ATAAAGGAGT ACCTCGCAGA ATGCATGGAT GCTACCCTTA CAATCCCCGT AGTTGCATAT 780  
 GAGATCCGTG ACTTTTGTAGA AGTTTCAGCA AAGCTTAAGG AGGATCATGC TGACCTGTTC 840  
 CCGTTTCTGG GGGCCATTAG ACACCCCGAC GCTATCAAGC TGGCGCCACG AAGETTTCCC 900  
 AATCTGGCCT CCGCAGCGTT TTAAGTGGAGT AAGAAGGAAA ACCCCACAAT GGCAGGETAC 960  
 CGGGCCTCCA CCATCCAGCC GGGCGCAAGT GTCAAGGAAA CCCAGETTCG CCGGTATAGG 1020  
 CGCCGCGAGA TATCTCGTGG AGAGGACGGG GCAGAGCTCT CAGGTGAGAT CTCTGCCATA 1080  
 ATGAAGATGA TAGGTGTGAC TGGTCTAAAC TA 1112

## (3) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 370 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Pro Lys Arg Arg Leu Val Asp Asp Ala Asp Ala Met Glu Asp  
 1 5 10 15  
 Gln Asp Leu Tyr Glu Pro Pro Ala Ser Leu Pro Lys Leu Pro Gly Lys  
 20 25 30  
 Phe Leu Gln Tyr Thr Val Gly Gly Ser Asp Pro His Pro Gly Ile Gly  
 35 40 45  
 His Glu Lys Asp Ile Arg Gln Asn Ala Val Ala Leu Leu Asp Gln Ser  
 50 55 60  
 Arg Arg Asp Met Phe His Thr Val Thr Pro Ser Leu Val Phe Leu Cys  
 65 70 75 80  
 Leu Leu Ile Pro Gly Leu His Ala Ala Phe Val His Gly Gly Val Pro  
 85 90 95  
 Arg Glu Ser Tyr Leu Ser Thr Pro Val Thr Arg Gly Glu Gln Thr Val  
 100 105 110  
 Val Lys Thr Ala Lys Phe Tyr Gly Glu Lys Thr Thr Gln Arg Asp Leu  
 115 120 125  
 Thr Glu Leu Glu Ile Ser Ser Ile Phe Ser His Cys Cys Ser Leu Leu

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130	135	140
Ile Gly Val Val	Ile Gly Ser Ser Ser Lys	Ile Lys Ala Gly Ala Glu
145	150	155 160
Gln Ile Lys Lys Arg Phe Lys Thr Met Met Ala Ala Leu Asn Arg Pro	165 170	175
Ser His Gly Glu Thr Ala Thr Leu Leu Gln Met Phe Asn Pro His Glu	180 185	190
Ala Ile Asp Trp Ile Asn Gly Gln Pro Trp Val Gly Ser Phe Val Leu	195 200	205
Ser Leu Leu Thr Thr Asp Phe Glu Ser Pro Gly Lys Glu Phe Met Asp	210 215	220
Gln Ile Lys Leu Val Ala Ser Tyr Ala Gln Met Thr Thr Tyr Thr Thr	225 230	235 240
Ile Lys Glu Tyr Leu Ala Glu Cys Met Asp Ala Thr Leu Thr Ile Pro	245 250	255
Val Val Ala Tyr Glu Ile Arg Asp Phe Leu Glu Val Ser Ala Lys Leu	260 265	270
Lys Glu Asp His Ala Asp Leu Phe Pro Phe Leu Gly Ala Ile Arg His	275 280	285
Pro Asp Ala Ile Lys Leu Ala Pro Arg Ser Phe Pro Asn Leu Ala Ser	290 295	300
Ala Ala Phe Tyr Trp Ser Lys Lys Glu Asn Pro Thr Met Ala Gly Tyr	305 310	315 320
Arg Ala Ser Thr Ile Gln Pro Gly Ala Ser Val Lys Glu Thr Gln Leu	325 330	335
Ala Arg Tyr Arg Arg Arg Glu Ile Ser Arg Gly Glu Asp Gly Ala Glu	340 345	350
Leu Ser Gly Glu Ile Ser Ala Ile Met Lys Met Ile Gly Val Thr Gly	355 360	365
Leu Asn		
370		

## (4) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 609 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCAACGC GACCATCGAG TCTGGTCGAC TCCCTGGAGG ACGAAGAAGA TCCCCAGACA	60
CTACGACGGG AACGACCGGG GTCACCAAGA CCACGGAAGG TCCCAAGGAA TGCATTGACC	120
CAACCAAGTAG ACCAGCTCCT GAAGGACCTC AGGAAGAACC CCTCCATGAT CTCAGACCCA	180
GACCAGCGAA CCGGAAGGGA GCAGCTGTCG AATGATGAGC TAATCAAGAA GTTAGTGACG	240
GAGCTGGCCG AGAATAGCAT GATCGAGGCT GAGGAGGTGC GGGGCACTCT TGGAGACATC	300

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TCGGCTCGTA TCGAGGCAGG GTTTGAGTCC CTGTCCGCCC TCCAAGTGA AACCATCCAG 360  
 ACAGCTCAGC GGTGCGATCA CTCGACAGC ATCAGGATCC TCGGCGAGAA CATCAAGATA 420  
 CTAGATCGCT CCATGAAGAC AATGATGGAG ACAATGAAGC TCATGATGGA GAAGGTGGAT 480  
 CTCCTCTACG CATCAACCGC CGTTGGGACC TCTGCACCCA TGTGCCCCC CCATCCTGCA 540  
 CCTCCGCGCA TTTATCCCA GCTCCAAGT GCCCGACAA CGGATGAATG GGACATCATA 600  
 CCATAAAAA 609

## (5) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 201 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Thr Arg Pro Ser Ser Leu Val Asp Ser Leu Glu Asp Glu Glu  
 1 5 10 15  
 Asp Pro Gln Thr Leu Arg Arg Glu Arg Pro Gly Ser Pro Arg Pro Arg  
 20 25 30  
 Lys Val Pro Arg Asn Ala Leu Thr Gln Pro Val Asp Gln Leu Leu Lys  
 35 40 45  
 Asp Leu Arg Lys Asn Pro Ser Met Ile Ser Asp Pro Asp Gln Arg Thr  
 50 55 60  
 Gly Arg Glu Gln Leu Ser Asn Asp Glu Leu Ile Lys Lys Leu Val Thr  
 65 70 75 80  
 Glu Leu Ala Glu Asn Ser Met Ile Glu Ala Glu Glu Val Arg Gly Thr  
 85 90 95  
 Leu Gly Asp Ile Ser Ala Arg Ile Glu Ala Gly Phe Glu Ser Leu Ser  
 100 105 110  
 Ala Leu Gln Val Glu Thr Ile Gln Thr Ala Gln Arg Cys Asp His Ser  
 115 120 125  
 Asp Ser Ile Arg Ile Leu Gly Glu Asn Ile Lys Ile Leu Asp Arg Ser  
 130 135 140  
 Met Lys Thr Met Met Glu Thr Met Lys Leu Met Met Glu Lys Val Asp  
 145 150 155 160  
 Leu Leu Tyr Ala Ser Thr Ala Val Gly Thr Ser Ala Pro Met Leu Pro  
 165 170 175  
 Ser His Pro Ala Pro Pro Arg Ile Tyr Pro Gln Leu Pro Ser Ala Pro  
 180 185 190  
 Thr Thr Asp Glu Trp Asp Ile Ile Pro  
 195 200

## (6) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 428 base pairs  
 (B) TYPE: nucleic acid

125.

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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ATGAATTCAA AACATTCTTA TGTGGAGCTC AAGGACAAGG TAATCGTCCC TGGATGGCCC . 60
ACACTGATGC TTGAGATAGA CTTTGTAGGG GGGACTTCAC GGAACCAGTT CCTTAACATC 120
CCATTTCTTT CAGTGAAAGA GCCTCTGAG CTTCCACGCG AGAAGAAGTT GACCGACTAC 180
TTTACTATTG ACGTAGAACC AGCAGGTCAT TCCCTGGTCA ATATATACTT CCAGATTGAC 240
GACTTCTTGC TCCTAACT CAETCACTA TCTGTGTACA AGGACCCGAT TAGAAAATAC 300
ATGTTCTCTAC GCCTCAACAA GGACCAGAGC AAGCACGCAA TCAATGCAGC CTTCAATGTC 360
TTTTCTTATC GGCTTCGGAA CATTGGTGTG GGTCTCTCG GCCCGGACAT TCGATCTTCA 420
GGGCCTTA 428

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(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 142 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Asn Ser Lys His Ser Tyr Val Glu Leu Lys Asp Lys Val Ile Val
1      5      10      15
Pro Gly Trp Pro Thr Leu Met Leu Glu Ile Asp Phe Val Gly Gly Thr
20     25     30
Ser Arg Asn Gln Phe Leu Asn Ile Pro Phe Leu Ser Val Lys Glu Pro
35     40     45
Leu Gln Leu Pro Arg Glu Lys Lys Leu Thr Asp Tyr Phe Thr Ile Asp
50     55     60
Val Glu Pro Ala Gly His Ser Leu Val Asn Ile Tyr Phe Gln Ile Asp
65     70     75     80
Asp Phe Leu Leu Leu Thr Leu Asn Ser Leu Ser Val Tyr Lys Asp Pro
85     90     95
Ile Arg Lys Tyr Met Phe Leu Arg Leu Asn Lys Asp Gln Ser Lys His
100    105    110
Ala Ile Asn Ala Ala Phe Asn Val Phe Ser Tyr Arg Leu Arg Asn Ile
115    120    125
Gly Val Gly Pro Leu Gly Pro Asp Ile Arg Ser Ser Gly Pro
130    135    140

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(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1515 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGCAGCCTT CAATGTCCTT TCTATCGGC TTCGGAACAT TGGTGTGGT CCTCTCGGCC	60
CGGACATTCC ATCTTCAGGG CCTTAGTGC AATACTGACT CCACTCCTGG ACTGATTGAC	120
CTGGAGATAA GGCAGCTTTG CCACACCCCA ACGGAAAATG TCATTTTCATG CGAGGTAGT	180
TATCTCAACC ACACGACTAT TAGCCTCCCG GCAGTCCACA CATCATGCCT CAAGTACCAC	240
TGCAAAACCT ATTGGGGATT CTTTGGTAGC TACAGCGCTG ACCGAATCAT AAATCGGTAC	300
ACTGGTACTG TTAAGGGTTG TCTAAACAAC TCAGCACCAG AGGACCCCTT CGAGTGCAAC	360
TGGTTCTACT GCTGCTCGGC GATTACAACA GAGATCTGCC GATGCTCTAT TACAAATGTC	420
ACGGTGCGTG TGCAAAACATT CCCACCGTTC ATGTACTGCA GTTTTGAGA CTGCAGTACC	480
GTGAGCCAAC AGGAGCTAGA GAGTGGAAAG GCAATGCTGA GCGATGGCAG TACATTAAT	540
TATACCCCGT ATATCCTACA GTCAGAAGTC GTGAACAAAA CCCTCAATGG GACCATACTC	600
TGCAACTCAT CCTCTAAGAT AGTTTCCTTC GATGAATTTA GCGGTTTATA CTCCTAACG	660
AATGCTAGTT ACCAGAGCTC ATCAATCAAT GTGACGTGTG CAACTACAC GTCGCTCTGC	720
CGGCCAGGT TGAAGGGCG GCGTAGGGAC ACCCAGCAGA TTGAGTATCT AGTTCACAAG	780
CTTAGGCCCA CACTGAAAGA TGATGGGAG GACTGTGAGA TCCTCCAGTC TCTGCTCTA	840
GGGGTGTTTG GTACTGGGAT CGCAAGTGCT TCTCAATTTT TGAGGAGCTG GCTCAACCAC	900
CCTGACATCA TCGGGTATAT AGTTAATGGA GTTGGGGTTG TCTGCAATG CCATCGTGTT	960
AATGTCACGT TCATGGCGTG GAATGAGTCC ACCTATTACC CTCCAGTAGA TTACAATGGG	1020
CGGAAGTACT TCCTGAATGA TGAGGGAAGG TTACAAACAA ACACCCCCGA GGCAAGGCCA	1080
GGGCTTAAGC GGGTCATGTG GTTCGGCAGG TACTTCTAG GGACAGTAGG GTCTGGGGTG	1140
AAACCGAGGA GGATTCGGTA CAATAAGACC TCACATGACT ACCACCTGGA GGAGTTTGAG	1200
GCAAGTCTCA ACATGACCCC TCAGACCAGT ATCGCCTCGG GTCATGAGAC AGACCCATA	1260
AATCATGCCT ACGGAACGCA GGCTGATCTC CTTCATACA CCAGGTCTAG TAATATAACA	1320
TCTACGGATA CAGGCTCAGG CTGGGTGCAC ATCGGCCTAC CCTCATTGTC TTTCTCAAT	1380
CCCTCGGGT GGCTCAGGA CCTACTTGCA TGGGCAGCCT GGTGGGTGG GGTCTATAC	1440
TTAATAAGTC TTTGTGTTT CTTACCAGCC TCCTTCGCGA GGAGGAGACG CCTCGGCCGG	1500
TGGCAGGAAT AAAC	1515

(9) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 503 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein



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(x1) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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Met Gln Pro Ser Met Ser Phe Leu Ile Gly Phe Gly Thr Leu Val Leu
1           5           10           15

Val Leu Ser Ala Arg Thr Phe Asp Leu Gln Gly Leu Ser Cys Asn Thr
20           25           30

Asp Ser Thr Pro Gly Leu Ile Asp Leu Glu Ile Arg Arg Leu Cys His
35           40           45

Thr Pro Thr Glu Asn Val Ile Ser Cys Glu Val Ser Tyr Leu Asn His
50           55           60

Thr Thr Ile Ser Leu Pro Ala Val His Thr Ser Cys Leu Lys Tyr His
65           70           75           80

Cys Lys Thr Tyr Trp Gly Phe Phe Gly Ser Tyr Ser Ala Asp Arg Ile
85           90           95

Ile Asn Arg Tyr Thr Gly Thr Val Lys Gly Cys Leu Asn Asn Ser Ala
100          105          110

Pro Glu Asp Pro Phe Glu Cys Asn Trp Phe Tyr Cys Cys Ser Ala Ile
115          120          125

Thr Thr Glu Ile Cys Arg Cys Ser Ile Thr Asn Val Thr Val Ala Val
130          135          140

Gln Thr Phe Pro Pro Phe Met Tyr Cys Ser Phe Ala Asp Cys Ser Thr
145          150          155          160

Val Ser Gln Gln Glu Leu Glu Ser Gly Lys Ala Met Leu Ser Asp Gly
165          170          175

Ser Thr Leu Thr Tyr Thr Pro Tyr Ile Leu Gln Ser Glu Val Val Asn
180          185          190

Lys Thr Leu Asn Gly Thr Ile Leu Cys Asn Ser Ser Ser Lys Ile Val
195          200          205

Ser Phe Asp Glu Phe Arg Arg Ser Tyr Ser Leu Thr Asn Gly Ser Tyr
210          215          220

Gln Ser Ser Ser Ile Asn Val Thr Cys Ala Asn Tyr Thr Ser Ser Cys
225          230          235          240

Arg Pro Arg Leu Lys Arg Arg Arg Arg Asp Thr Gln Gln Ile Glu Tyr
245          250          255

Leu Val His Lys Leu Arg Pro Thr Leu Lys Asp Ala Trp Glu Asp Cys
260          265          270

Glu Ile Leu Gln Ser Leu Leu Leu Gly Val Phe Gly Thr Gly Ile Ala
275          280          285

Ser Ala Ser Gln Phe Leu Arg Ser Trp Leu Asn His Pro Asp Ile Ile
290          295          300

Gly Tyr Ile Val Asn Gly Val Gly Val Val Trp Gln Cys His Arg Val
305          310          315          320

Asn Val Thr Phe Met Ala Trp Asn Glu Ser Thr Tyr Tyr Pro Pro Val
325          330          335

Asp Tyr Asn Gly Arg Lys Tyr Phe Leu Asn Asp Glu Gly Arg Leu Gln
340          345          350

Thr Asn Thr Pro Glu Ala Arg Pro Gly Leu Lys Arg Val Met Trp Phe
355          360          365

Gly Arg Tyr Phe Leu Gly Thr Val Gly Ser Gly Val Lys Pro Arg Arg
370          375          380

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Ile Arg Tyr Asn Lys Thr Ser His Asp Tyr His Leu Glu Glu Phe Glu  
385 390 395 400

Ala Ser Leu Asn Met Thr Pro Gln Thr Ser Ile Ala Ser Gly His Glu  
405 410 415

Thr Asp Pro Ile Asn His Ala Tyr Gly Thr Gln Ala Asp Leu Leu Pro  
420 425 430

Tyr Thr Arg Ser Ser Asn Ile Thr Ser Thr Asp Thr Gly Ser Gly Trp  
435 440 445

Val His Ile Gly Leu Pro Ser Phe Ala Phe Leu Asn Pro Leu Gly Trp  
450 455 460

Leu Arg Asp Leu Leu Ala Trp Ala Ala Trp Leu Gly Gly Val Leu Tyr  
465 470 475 480

Leu Ile Ser Leu Cys Val Ser Leu Pro Ala Ser Phe Ala Arg Arg Arg  
485 490 495

Arg Leu Gly Arg Trp Gln Glu  
500

## (10) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5135 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGTCATTTC ATCGGAGCCT CCTTCGCGAG GAGGAGACGC CTCGGCCGGT GGCAGGAATA	60
AACCGTACCG ACCAGTCTCT TAAAAACCT CTCCTCGGAA CAGAGGTCTC TTTCTGCCTT	120
AAGTCGAGCT CACTCCCCCA TCATGTACGA GCACTAGGCC AGATTAAAGC AAGGAACCTG	180
GCATCCTGTG ACTATTACTT GCTATTCGCG CAAGTTGTAT TGCCCCCTGA AGTATATCCC	240
ATTGGTGTTC TAATAAGAGC TCGGAGGCT ATACTAACAG TTATAGTATC AGCTTGAAG	300
CTGGATCATA TGACGAAGAC CCTATACTCC TCTGTGAGAT ATGCACTCAC CAATCCCCGG	360
GTCCGAGCCC AACTTGAGCT TCACATTGCC TACCAGCGCA TAGTGGGTCA GGTCTCGTAC	420
AGCCGGGAGG CAGACATAGG GCCAAAAGG CTTGGGAATA TGTCATTGCA ATTCATCCAA	480
TCTCTCGTTA TTGCCACCAT AGACACGACA AGCTGCCTAA TGACCTACAA CCACTTTCTT	540
GCTGCAGCAG ACACAGCCAA GAGCAGATGC CATCTCCTAA TCGCCTCAGT GGTCCAGGGG	600
GCCCTTTGGG AACAGGGTC ATTTCTTGAT CATATAATCA ACATGATCGA CATAATTGAC	660
TCAATCAACC TCCCCATGA TGATTACTTC ACAATTATTA AGTCTATCTT TCCCTACTCC	720
CAAGGGCTTG TTATGGGGAG GCATAATGTA TCAGTCTCCT CTGATTTGCG GTCCGTATTT	780
GCCATTCTCG AATTATGCCC GCAACTAGAC AGETTACTAA AAAAAGTCT CCAACTTGAC	840
CCCGTTCTCC TCCTCATGGT CTCTTCGGTG CAGAAGTCAT GGTACTTCCC TGAGATCCGA	900
ATGGTCGACG GGTACGGGA GCAGCTCCAC AAGATGCGTG TCGAGETGGA AACGCCCCAA	960
GCCCTGCTGT CGTACGGCCA TACCCTCTG TCAATATTTT GGCAGAGTT TATCAAAGGC	1020

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TATGTCTCAA AGAATGCGAA GTGGCCGCCC GTACACCTGC TCCCAGGCTG TGACAAATCC	1080
ATAAAAAATG CGAGAGAGCT GGGCCGCTGG AGCCCGGCAT TTGACCGACG ATGGCAGCTC	1140
TTGAGAAGG TTGTCAATTCT AAGAATTGCT GACCTAGATA TGGATCCGA CTTCAACGAT	1200
ATTGTTAGCG ATAAGGCGAT AATCAGCTCA AGAAGGGACT GGGTATTGCA GTACAATGCA	1260
GCGGCCTTTT GGAAGAAATA CGGTGAACGG TTGGAGAGGC CTCCTGCCAG GTCGGGACCG	1320
TCACGACTTG TGAATGCTCT AATCGATGGA CGCTTAGACA ATATCCAGC CCTGCTAGAG	1380
CCATTTTACA GGGGAGCGGT TGAGTTCGAG GATCGGTTGA CTGTGCTCGT GCCTAAGGAG	1440
AAAGAGTTAA AGGTAAAGG AAGGTTCTTC TCGAAGCAAA CATTGGCAAT CAGGATATAT	1500
CAGGTTGTTG CTGAAGTGC ACTTAAGAAT GAGGTTATGC CATACCTAAA GACACACTCA	1560
ATGACCATGA GCTCAACGGC TCTAACTCAC CTTCTTAACC GGCTATCACA TACTATCACT	1620
AAGGGTGACT CTTTGTGTAT TAACCTTGAC TATAGTTCCG GGTGCAACGG TTTCCGACCA	1680
GAACTGCAGG CCCCAATCTG TCGTCAGTTG GATCAGATGT TCAATTGCGG GTAATTCTTC	1740
AGGACTGGGT GCACACTGCC ATGCTTTACC ACGTTTATTA TTCAAGACAG GTTCAACCCG	1800
CCCTATTCCC TCAGTGCTGA GCCCCTTGAA GACGGAGTTA CATGCGCGGT TGGGACTAAA	1860
ACAATGGGGG AGGGCATGAG GCAGAACTA TGGACAATCC TTACGAGCTG CTGGGAGATA	1920
ATTGCTCTTC GGGAAATTA CGTGACGTTT AACATACTAG GCCAAGGTGA TAATCAGACA	1980
ATCATCATAC ATAAATCTGC AAGCCAAAAT AACCACTAT TAGCGGAGCG AGCACTAGGG	2040
GCCCTGTACA AGCATGCTAG ATTAGCTGSC CATAACCTCA AGGTAGAGGA ATGCTGGGTG	2100
TCGATTGTC TGTATGAGTA TGGAAAGAAG CTTTCTCTCC GTGGTGATCC TGTCGCGGCG	2160
TGTTTGAGC AGCTCTCAGC GGTGACGGAT TCTACTGGAG AGCTATTCCC AAACCTATAC	2220
TCAAAGTTAG CCGTCTTAAC ATCATCGTGT TTAAGCGCAG CGATGGCAGA CACATCTCCA	2280
TGGGTGGCAG TCGCGACAGG TGTCTGTCTG TATCTTATCG AGTTATATGT TGAGCTGCCT	2340
CCAGCAATCA TGCAGGATGA GTCGCTATTG ACGACCTCT GCTCGTAGG CCCATCCATT	2400
GGTGGGCTTC CGACCCCTGC AACCTACCC AGTGCTCTTT TCAGAGGAAT GTCGACCCA	2460
CTGCCCTTTC AGCTAGCACT CTTGCAGACC CTCATTAAGA CGACAGGGGT GACCTGTAGC	2520
TTGGTGAATC GTGTGGTCAA GTTACGGATA GCACCTATC CAGACTGGCT CTCTCTAGTG	2580
ACTGACCCGA CCTCACTCAA CATTGCCCAA GTGTACCGGC CAGAAGTCA GATCAGGAGG	2640
TGGATTGAGG AAGCGATAGC GACAAGCTCA CACTCGTCAC GCATAGCAAC TTTCTTCCAG	2700
CAGCCCCCTA CGGAGATGCG TCAGTTGCTT GCGAGGGACC TTTCAACAAAT GATGCCTCTT	2760
CGACCCCGGG ATATGTCGGC CTTATTGCGA TTATCAAAATG TCGCATACGG TTTAAGCATT	2820
ATAGATCTAT TTCAAAAATC CTCTACCGTT GTTTCTGCAA GTCAAGCTGT CCATATCGAG	2880
GATGTTGCCC TAGAGAGTGT AAGGTATAAG GAATCTATCA TCCAGGCTCT GTTAGACACC	2940
ACTGAGGGGT ATAACATGCA ACCTTATTG GAAGGTTGCA CTTACCTTGC AGCCAAACAG	3000
TTACGTAGGT TGACATGGGG TCGAGACCTA GTTGGAGTCA CAATGCCGTT TGTGCGCGAG	3060
CAATTCATC CTCACASTTC TGTGGGTGCA AAGGCGGAAC TCTACCTCGA CGCTATTATA	3120
TACTGCCAC AGGAGACATT GCGGTCACAC CATCTGACTA CCAGGGGGGA CCAGCCGCTT	3180
TACCTCGGAT CCAATACGGC TGTCAAGGTC CAGCGAGGTG AGATCACGGG CCTAACAAAG	3240

TCAAGGGCTG CAAATCTAGT CAGGGACACT CTCGTTCTCC ATCAGTGGTA TAAAGTCCGT	3300
AAAGTTACCG ATCCACACTT GAACACCCCTC ATGGCACGCT TCTTACTTGA GAAGGGGTAC	3360
ACATCTGACG CTCGACCTAG CATCCAGGCT GGGACCCTCA CGCATCGTCT CCCATCCCGC	3420
GGAGACTCAC GGCAGGGGCT TACTGGGTAT GTAAATATAC TAAGTACGTG GCTTCGATTG	3480
TCAAGTGATT ATCTTCACTC TTTCTCGAAA TCATCAGACG ACTATACAAT CCACTTTCAG	3540
CATGTATTCA CATACGGTTG CCTCTATGCT GATTCGGTGA TTAGATCGGG CGGTGTTATT	3600
TCCACTCCTT ACCTTTTGAG TGCAAGTTGT AAAACATGCT TTGAGAAGAT AGACTCAGAG	3660
GAGTTCGTCC TGGCATGTGA ACCCAATAC AGGGGTGCTG AGTGGCTGAT ATCAAAGCCA	3720
GTCAGTGTCC CTGAGCAGAT AACTGATGCT GAAGTCGAGT TTGACCCCTG TGTGAGTGCG	3780
GGTTATTGTC TCGGGATTCT CATTGGCAAG TCATTCTTAG TTGACATAAG GGCAAGTGGG	3840
CATGATATCA TGGAGCAGCG GACATGGGCT AACCTGGAGA GGTTTTCTGT ATCGGACATG	3900
CAGAAACTTC CGTGGAGTAT TGTAAATCGG TCTCTCTGGA GATTCCTTAT TGEGGACGG	3960
CTCCTTCAGT TTGAGAAGGC TGGCCTCATT AGAATGCTGT ATGCTGCGAC AGSTCCAACC	4020
CCTAGCTTCC TAATGAAAGT TTTTCAAGAC TCAGCCCTCC TCATGGACTG CGACCCCTC	4080
GATCGGCTGT CCCCTAGGAT CAACTTTCAT AGTCGGGGAG ACCTCGTTGC TAAGCTTGTT	4140
TTATTGCCCT TCATCAACCC GGGTATAGTG GAGATTGAAG TGTCTGGAAT TAATAGCAAG	4200
TACCATGCAG TATCGEAGGC CAATATGGAT CTGTACATCG CTGCTGCCAA GTCTGTGGGC	4260
GTGAAGCCCA CACAGTTTGT TGAGGAAACA AACGACTTTA CGGCCCGCGG CCACCACCAT	4320
GGTTGTTATT CCGTTTCTTG GTCTAAGTCA CGCAATCAAT CACAGTCTCT AAAGATGGTA	4380
GTACGGAAGC TGAAGCTCTG TGTCTCTGAT ATATACCCCA CAGTCGATCC CGCCGTTGCT	4440
CTCGACCTGT GCCATCTACC AGCATTAACT ATAATCCTAG TGCTCGGCGG TGACCCAGCG	4500
TACTATGAGC GATTACTTGA GATGGACCTG TCGGGGGCTG TGTCAAGTCG AGTCGATATC	4560
CCCCATTCTC TGGCTGGCAG AACGCACAGG GGETTCGCAG TGGGCCCAGA CGCTGGTCCA	4620
GGTGTAATTA GACTCGACAG GTTAGAGTCA GTTTGTTATG CTCACCCCTG TTTAGAGGAA	4680
CTAGAGTTTA ATGCATATCT AGACTCTGAG TTGGTTGACA TTAGTGATAT GTGCTGCCTC	4740
CCCTTAGCGA CACCCTGTAA GGGCCCTTTC AGGCCAATAT ATCGGAGCTT ACAGTCGTTT	4800
AGGTTAGCCT TAATGGACAA CTATAGTTTT GTCATGGACC TCATTATGAT CCGAGGACTG	4860
GACATTAGGC CTCACCTTGA GGAATTTGAC GAGCTGCTTG TGGTAGGACA GCACATCCTC	4920
GGCCAGCCCG TCCTAGTAGA GGTGTTTAC TACGTTGGAG TTGTTAGGAA GCGCCCTGTG	4980
TTAGCGAGGC ATCCGTGGTC AGCAGATCTT AAGCGAATTA CTGTGGGGGG GCGGGCTCCC	5040
TGCCCTCTG CTGCCAGATT GCGTGATGAG GATTGTCAGG GGTCTCTGTT GGTGCGGCTT	5100
CCTGCTGGGT TGACGCAGTT ATTGATAATT GATTA	5135

## (11) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1711 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

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(x1) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Phe His Ala Ser Leu Leu Arg Glu Glu Glu Thr Pro Arg Pro  
1 5 10 15  
Val Ala Gly Ile Asn Arg Thr Asp Gln Ser Leu Lys Asn Pro Leu Leu  
20 25 30  
Gly Thr Glu Val Ser Phe Cys Leu Lys Ser Ser Ser Leu Pro His His  
35 40 45  
Val Arg Ala Leu Gly Gln Ile Lys Ala Arg Asn Leu Ala Ser Cys Asp  
50 55 60  
Tyr Tyr Leu Leu Phe Arg Gln Val Val Leu Pro Pro Glu Val Tyr Pro  
65 70 75 80  
Ile Gly Val Leu Ile Arg Ala Ala Glu Ala Ile Leu Thr Val Ile Val  
85 90 95  
Ser Ala Trp Lys Leu Asp His Met Thr Lys Thr Leu Tyr Ser Ser Val  
100 105 110  
Arg Tyr Ala Leu Thr Asn Pro Arg Val Arg Ala Gln Leu Glu Leu His  
115 120 125  
Ile Ala Tyr Gln Arg Ile Val Gly Gln Val Ser Tyr Ser Arg Glu Ala  
130 135 140  
Asp Ile Gly Pro Lys Arg Leu Gly Asn Met Ser Leu Gln Phe Ile Gln  
145 150 155 160  
Ser Leu Val Ile Ala Thr Ile Asp Thr Thr Ser Cys Leu Met Thr Tyr  
165 170 175  
Asn His Phe Leu Ala Ala Ala Asp Thr Ala Lys Ser Arg Cys His Leu  
180 185 190  
Leu Ile Ala Ser Val Val Gln Gly Ala Leu Trp Glu Gln Gly Ser Phe  
195 200 205  
Leu Asp His Ile Ile Asn Met Ile Asp Ile Ile Asp Ser Ile Asn Leu  
210 215 220  
Pro His Asp Asp Tyr Phe Thr Ile Ile Lys Ser Ile Phe Pro Tyr Ser  
225 230 235 240  
Gln Gly Leu Val Met Gly Arg His Asn Val Ser Val Ser Ser Asp Phe  
245 250 255  
Ala Ser Val Phe Ala Ile Pro Glu Leu Cys Pro Gln Leu Asp Ser Leu  
260 265 270  
Leu Lys Lys Leu Leu Gln Leu Asp Pro Val Leu Leu Leu Met Val Ser  
275 280 285  
Ser Val Gln Lys Ser Trp Tyr Phe Pro Glu Ile Arg Met Val Asp Gly  
290 295 300  
Ser Arg Glu Gln Leu His Lys Met Arg Val Glu Leu Glu Thr Pro Gln  
305 310 315 320  
Ala Leu Leu Ser Tyr Gly His Thr Leu Leu Ser Ile Phe Arg Ala Glu  
325 330 335  
Phe Ile Lys Gly Tyr Val Ser Lys Asn Ala Lys Trp Pro Pro Val His  
340 345 350  
Leu Leu Pro Gly Cys Asp Lys Ser Ile Lys Asn Ala Arg Glu Leu Gly  
355 360 365  
Arg Trp Ser Pro Ala Phe Asp Arg Arg Trp Gln Leu Phe Glu Lys Val  
370 375 380

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Val Ile Leu Arg Ile Ala Asp Leu Asp Met Asp Pro Asp Phe Asn Asp  
 385 390 395 400  
 Ile Val Ser Asp Lys Ala Ile Ile Ser Ser Arg Arg Asp Trp Val Phe  
 405 410 415  
 Glu Tyr Asn Ala Ala Phe Trp Lys Lys Tyr Gly Glu Arg Leu Glu  
 420 425 430  
 Arg Pro Pro Ala Arg Ser Gly Pro Ser Arg Leu Val Asn Ala Leu Ile  
 435 440 445  
 Asp Gly Arg Leu Asp Asn Ile Pro Ala Leu Leu Glu Pro Phe Tyr Arg  
 450 455 460  
 Gly Ala Val Glu Phe Glu Asp Arg Leu Thr Val Leu Val Pro Lys Glu  
 465 470 475 480  
 Lys Glu Leu Lys Val Lys Gly Arg Phe Phe Ser Lys Gln Thr Leu Ala  
 485 490 495  
 Ile Arg Ile Tyr Gln Val Val Ala Glu Ala Ala Leu Lys Asn Glu Val  
 500 505 510  
 Met Pro Tyr Leu Lys Thr His Ser Met Thr Met Ser Ser Thr Ala Leu  
 515 520 525  
 Thr His Leu Leu Asn Arg Leu Ser His Thr Ile Thr Lys Gly Asp Ser  
 530 535 540  
 Phe Val Ile Asn Leu Asp Tyr Ser Ser Trp Cys Asn Gly Phe Arg Pro  
 545 550 555 560  
 Glu Leu Gln Ala Pro Ile Cys Arg Gln Leu Asp Gln Met Phe Asn Cys  
 565 570 575  
 Gly Tyr Phe Phe Arg Thr Gly Cys Thr Leu Pro Cys Phe Thr Thr Phe  
 580 585 590  
 Ile Ile Gln Asp Arg Phe Asn Pro Pro Tyr Ser Leu Ser Gly Glu Pro  
 595 600 605  
 Val Glu Asp Gly Val Thr Cys Ala Val Gly Thr Lys Thr Met Gly Glu  
 610 615 620  
 Gly Met Arg Gln Lys Leu Trp Thr Ile Leu Thr Ser Cys Trp Glu Ile  
 625 630 635 640  
 Ile Ala Leu Arg Glu Ile Asn Val Thr Phe Asn Ile Leu Gly Gln Gly  
 645 650 655  
 Asp Asn Gln Thr Ile Ile Ile His Lys Ser Ala Ser Gln Asn Asn Gln  
 660 665 670  
 Leu Leu Ala Glu Arg Ala Leu Gly Ala Leu Tyr Lys His Ala Arg Leu  
 675 680 685  
 Ala Gly His Asn Leu Lys Val Glu Glu Cys Trp Val Ser Asp Cys Leu  
 690 695 700  
 Tyr Glu Tyr Gly Lys Lys Leu Phe Phe Arg Gly Val Pro Val Pro Gly  
 705 710 715 720  
 Cys Leu Lys Gln Leu Ser Arg Val Thr Asp Ser Thr Gly Glu Leu Phe  
 725 730 735  
 Pro Asn Leu Tyr Ser Lys Leu Ala Cys Leu Thr Ser Ser Cys Leu Ser  
 740 745 750  
 Ala Ala Met Ala Asp Thr Ser Pro Trp Val Ala Leu Ala Thr Gly Val  
 755 760 765  
 Cys Leu Tyr Leu Ile Glu Leu Tyr Val Glu Leu Pro Pro Ala Ile Met  
 770 775 780

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Gln Asp Glu Ser Leu Leu Thr Thr Leu Cys Leu Val Gly Pro Ser Ile  
 785 790 795 800  
 Gly Gly Leu Pro Thr Pro Ala Thr Leu Pro Ser Val Phe Phe Arg Gly  
 805 810 815  
 Met Ser Asp Pro Leu Pro Phe Gln Leu Ala Leu Leu Gln Thr Leu Ile  
 820 825 830  
 Lys Thr Thr Gly Val Thr Cys Ser Leu Val Asn Arg Val Val Lys Leu  
 835 840 845  
 Arg Ile Ala Pro Tyr Pro Asp Trp Leu Ser Leu Val Thr Asp Pro Thr  
 850 855 860  
 Ser Leu Asn Ile Ala Gln Val Tyr Arg Pro Glu Arg Gln Ile Arg Arg  
 865 870 875 880  
 Trp Ile Glu Glu Ala Ile Ala Thr Ser Ser His Ser Ser Arg Ile Ala  
 885 890 895  
 Thr Phe Phe Gln Gln Pro Leu Thr Glu Met Ala Gln Leu Leu Ala Arg  
 900 905 910  
 Asp Leu Ser Thr Met Met Pro Leu Arg Pro Arg Asp Met Ser Ala Leu  
 915 920 925  
 Phe Ala Leu Ser Asn Val Ala Tyr Gly Leu Ser Ile Ile Asp Leu Phe  
 930 935 940  
 Gln Lys Ser Ser Thr Val Val Ser Ala Ser Gln Ala Val His Ile Glu  
 945 950 955 960  
 Asp Val Ala Leu Glu Ser Val Arg Tyr Lys Glu Ser Ile Ile Gln Gly  
 965 970 975  
 Leu Leu Asp Thr Thr Glu Gly Tyr Asn Met Gln Pro Tyr Leu Glu Gly  
 980 985 990  
 Cys Thr Tyr Leu Ala Ala Lys Gln Leu Arg Arg Leu Thr Trp Gly Arg  
 995 1000 1005  
 Asp Leu Val Gly Val Thr Met Pro Phe Val Ala Glu Gln Phe His Pro  
 1010 1015 1020  
 His Ser Ser Val Gly Ala Lys Ala Glu Leu Tyr Leu Asp Ala Ile Ile  
 1025 1030 1035 1040  
 Tyr Cys Pro Gln Glu Thr Leu Arg Ser His His Leu Thr Thr Arg Gly  
 1045 1050 1055  
 Asp Gln Pro Leu Tyr Leu Gly Ser Asn Thr Ala Val Lys Val Gln Arg  
 1060 1065 1070  
 Gly Glu Ile Thr Gly Leu Thr Lys Ser Arg Ala Ala Asn Leu Val Arg  
 1075 1080 1085  
 Asp Thr Leu Val Leu His Gln Trp Tyr Lys Val Arg Lys Val Thr Asp  
 1090 1095 1100  
 Pro His Leu Asn Thr Leu Met Ala Arg Phe Leu Leu Glu Lys Gly Tyr  
 1105 1110 1115 1120  
 Thr Ser Asp Ala Arg Pro Ser Ile Gln Gly Gly Thr Leu Thr His Arg  
 1125 1130 1135  
 Leu Pro Ser Arg Gly Asp Ser Arg Gln Gly Leu Thr Gly Tyr Val Asn  
 1140 1145 1150  
 Ile Leu Ser Thr Trp Leu Arg Phe Ser Ser Asp Tyr Leu His Ser Phe  
 1155 1160 1165  
 Ser Lys Ser Ser Asp Asp Tyr Thr Ile His Phe Gln His Val Phe Thr  
 1170 1175 1180

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Tyr Gly Cys Leu Tyr Ala Asp Ser Val Ile Arg Ser Gly Gly Val Ile  
 1185 1190 1195 1200  
 Ser Thr Pro Tyr Leu Leu Ser Ala Ser Cys Lys Thr Cys Phe Glu Lys  
 1205 1210 1215  
 Ile Asp Ser Glu Glu Phe Val Leu Ala Cys Glu Pro Gln Tyr Arg Gly  
 1220 1225 1230  
 Ala Glu Trp Leu Ile Ser Lys Pro Val Thr Val Pro Glu Gln Ile Thr  
 1235 1240 1245  
 Asp Ala Glu Val Glu Phe Asp Pro Cys Val Ser Ala Gly Tyr Cys Leu  
 1250 1255 1260  
 Gly Ile Leu Ile Gly Lys Ser Phe Leu Val Asp Ile Arg Ala Ser Gly  
 1265 1270 1275 1280  
 His Asp Ile Met Glu Gln Arg Thr Trp Ala Asn Leu Glu Arg Phe Ser  
 1285 1290 1295  
 Val Ser Asp Met Gln Lys Leu Pro Trp Ser Ile Val Ile Arg Ser Leu  
 1300 1305 1310  
 Trp Arg Phe Leu Ile Gly Ala Arg Leu Leu Gln Phe Glu Lys Ala Gly  
 1315 1320 1325  
 Leu Ile Arg Met Leu Tyr Ala Ala Thr Gly Pro Thr Pro Ser Phe Leu  
 1330 1335 1340  
 Met Lys Val Phe Gln Asp Ser Ala Leu Leu Met Asp Cys Ala Pro Leu  
 1345 1350 1355 1360  
 Asp Arg Leu Ser Pro Arg Ile Asn Phe His Ser Arg Gly Asp Leu Val  
 1365 1370 1375  
 Ala Lys Leu Val Leu Leu Pro Phe Ile Asn Pro Gly Ile Val Glu Ile  
 1380 1385 1390  
 Glu Val Ser Gly Ile Asn Ser Lys Tyr His Ala Val Ser Glu Ala Asn  
 1395 1400 1405  
 Met Asp Leu Tyr Ile Ala Ala Ala Lys Ser Val Gly Val Lys Pro Thr  
 1410 1415 1420  
 Gln Phe Val Glu Glu Thr Asn Asp Phe Thr Ala Arg Gly His His His  
 1425 1430 1435 1440  
 Gly Cys Tyr Ser Leu Ser Trp Ser Lys Ser Arg Asn Gln Ser Gln Val  
 1445 1450 1455  
 Leu Lys Met Val Val Arg Lys Leu Lys Leu Cys Val Leu Tyr Ile Tyr  
 1460 1465 1470  
 Pro Thr Val Asp Pro Ala Val Ala Leu Asp Leu Cys His Leu Pro Ala  
 1475 1480 1485  
 Leu Thr Ile Ile Leu Val Leu Gly Gly Asp Pro Ala Tyr Tyr Glu Arg  
 1490 1495 1500  
 Leu Leu Glu Met Asp Leu Cys Gly Ala Val Ser Ser Arg Val Asp Ile  
 1505 1510 1515 1520  
 Pro His Ser Leu Ala Gly Arg Thr His Arg Gly Phe Ala Val Gly Pro  
 1525 1530 1535  
 Asp Ala Gly Pro Gly Val Ile Arg Leu Asp Arg Leu Glu Ser Val Cys  
 1540 1545 1550  
 Tyr Ala His Pro Cys Leu Glu Glu Leu Glu Phe Asn Ala Tyr Leu Asp  
 1555 1560 1565  
 Ser Glu Leu Val Asp Ile Ser Asp Met Cys Cys Leu Pro Leu Ala Thr  
 1570 1575 1580



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Pro Cys Lys Ala Leu Phe Arg Pro Ile Tyr Arg Ser Leu Gln Ser Phe  
1585 1590 1595 1600

Arg Leu Ala Leu Met Asp Asn Tyr Ser Phe Val Met Asp Leu Ile Met  
1605 1610 1615

Ile Arg Gly Leu Asp Ile Arg Pro His Leu Glu Glu Phe Asp Glu Leu  
1620 1625 1630

Leu Val Val Gly Gln His Ile Leu Gly Gln Pro Val Leu Val Glu Val  
1635 1640 1645

Val Tyr Tyr Val Gly Val Val Arg Lys Arg Pro Val Leu Ala Arg His  
1650 1655 1660

Pro Trp Ser Ala Asp Leu Lys Arg Ile Thr Val Gly Gly Arg Ala Pro  
1665 1670 1675 1680

Cys Pro Ser Ala Ala Arg Leu Arg Asp Glu Asp Cys Gln Gly Ser Leu  
1685 1690 1695

Leu Val Gly Leu Pro Ala Gly Leu Thr Gln Leu Leu Ile Ile Asp  
1700 1705 1710

## (12) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCTCCCCCTT AGCGACACCC TGTA

24

## (13) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GAAACATATC GCGCCGTGCA

20

## (14) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 22 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TACGTTGGAG TTGTTAGGAA GC

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(15) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGCTTAGGG AGGCTCGCTG

20

(16) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCCTCGAGAT GAATCAAAA CATTCTATC

30

(17) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTAAGGCCCT GAAGATCGAA T

21

(18) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCCTCGAGGA CCAAGATT

19

(19) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGAATCATAT GGCAACGCGA CCATC

25

(20) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8910 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTTGCGTTAA CAACAACCA CTCATCATT	60
CCAAGAGACG CCTGTTGAT GACGCCGATG	120
CAGCGAGCCT CCCTAAGCTC CCTGGGAAAT	180
CGCATCCGGG TATAGGGCAT GAGAAAGACA	240
AGTCACGGCG CGATATGTTT CACACAGTAA	300
TCCCAGGACT GCACGCTGCG TTTGTTACG	360
CGCCTGTAC GCGTGAGAA CAGACTGTTG	420
CGACGCAGCG TGATCTCACC GAGCTGGAGA	480
TACTAATAGG GGTGTGATA GGATCGTCGT	540
AGAAAAGGTT TAAACTATG ATGGCAGCCT	600
CACACTCCA GATGTTTAAT CCATGAGG	660
TAGGCTCCTT TGTGTTGCT CTACTAACTA	720
TGGACCAGAT TAAGCTTGC GCAAGTTATG	780
AGTACCTCGC AGAATGCATG GATGTACCC	840

GTGACTTTTT AGAAGTTTCA GCAAAGCTTA AGGAGGATCA TGCTGACCTG TTCCCGTTTC	900
TGGGGGCCAT TAGACACCCC GACGCTATCA AGCTGGCGCC ACGAAGCTTT CCCAATCTGG	960
CCTCCGCAGC GTTTTACTGG AGTAAGAAGG AAAACCCAC AATGGCAGGC TACCGGGCCT	1020
CCACCATCCA GCCGGGCGCA AGTGTCAAGG AAACCCAGCT TGCCCGGTAT AGGCGCCGCG	1080
AGATATCTCG TGGAGAGGAC GGGGCAGAGC TCTCAGGTGA GATCTCTGCC ATAATGAAGA	1140
TGATAGGTGT GACTGGTCTA AACTAAAAA CAATGAACAA ACCAATAAAA AACCAAATGC	1200
GGCAAACCTT CCGCGACCTG CGATGAGCTC CGACCTCCGG CTGACATTGC TTGAACTAGT	1260
CAGGAGGCTC AATGGCAACG CGACCATCGA GTCTGGTCGA CTCCTGGAG GACGAAGAAG	1320
ATCCCCAGAC ACTACGACGG GAACGACCGG GGTCAACCAAG ACCACGGAAG GTCCCAAGGA	1380
ATGCATTGAC CCAACCAGTA GACCAGCTCC TGAAGGACCT CAGGAAGAAC CCCTCCATGA	1440
TCTCAGACCC AGACCAGCGA ACCGGAAGGG AGCAGCTGTC GAATGATGAG CTAATCAAGA	1500
AGTTAGTGAC GGAGCTGGCC GAGAATAGCA TGATCGAGGC TGAGGAGGTG CGGGGCACTC	1560
TTGGAGACAT CTCGGCTCGT ATCGAGGCAG GGTTCGAGTC CCTGTCCGCC CTCCAAGTGG	1620
AAACCATCCA GACAGCTCAG CGGTGCGATC ACTCCGACAG CATCAGGATC CTCGGCGAGA	1680
ACATCAAGAT ACTAGATCGC TCCATGAAGA CAATGATGGA GACAATGAAG CTCATGATGG	1740
AGAAGGTGGA TCTCTCTAC GCATCAACCG CCGTTGGGAC CTCTGCACCC ATGTTGCCCT	1800
CCCATCTGTC ACCTCCGGCC ATTTATCCCC AGCTCCCAAG TGCCCCGACA ACGGATGAAT	1860
GGGACATCAT ACCATAAAAA AATCGAATCA CCATGAATTC AAAACATTCC TATGTGAGC	1920
TCAAGGACAA GGTAACTGTC CCTGGATGGE CCACACTGAT GCTTGAGATA GACTTTGTAG	1980
GGGGGACTTC ACGGAACCCAG TTCCTTAACA TCCCATTCTT TTCAGTGAAA GAGCCTCTGC	2040
AGCTTCCACG CGAGAAGAAG TTGACCGACT ACTTTACTAT TGACGTAGAA CCAGCAGGTC	2100
ATTCCCTGGT CAATATATAC TTCCAGATTG ACGACTTCTT GTCCTAACA CTCAACTCAC	2160
TATCTGTGTA CAAGGACCCG ATTAGAAAAT ACATGTTCTT ACGCCTCAAC AAGGACCAGA	2220
GCAAGCACGC AATCAATGCA GCCTTCAATG TCTTTTCTTA TCGGCTTCGG AACATTGGTG	2280
TTGGTCTCT CTGCCCCGAC ATTCGATCTT CAGGGCCTTA GCTGCAATAC TGACTCCACT	2340
CCTGGACTGA TTGACCTGGA GATAAGGCGA CTTTGCCACA CCCCACGGA AAATGTCATT	2400
TCATGCGAGG TTAGTTATCT CAACCAACAG ACTATTAGCC TCCCGGCAGT CCACACATCA	2460
TGCCTCAAGT ACCACTGCAA AACCTATTGG GGATTCTTTG GTAGCTACAG CGGTGACCGA	2520
ATCATAAATC GGTACACTGG TACTGTTAAG GGTGTCTAA ACAACTCAGC ACCAGAGGAC	2580
CCCTTCGAGT GCAACTGGTT CTACTGCTGC TCGGCGATTA CAACAGAGAT CTGCCGATGC	2640
TCTATTACAA ATGTCACGGT GGCTGTGCAA ACATTCCAC CGTTCATGTA CTGCAGTTTT	2700
GCAGACTGCA GTACCGTGAG CCAACAGGAG CTAGAGAGTG GAAAGGCAAT GCTGAGCGAT	2760
GGCAGTACAT TAACTTATAC CCCGTATATC CTACAGTCAG AAGTCGTGAA CAAAACCTC	2820
AATGGGACCA TACTGTGCAA CTCATCTCTT AAGATAGTTT CCTTCGATGA ATTTAGCGGT	2880
TCATACTCCC TAACGAATGG TAGTTACCAG AGCTCATCAA TCAATGTGAC GTGTGCAAAC	2940
TACACGTCGT CCTGCCGGCC CAGGTTGAAA AGGCGGCGTA GGGACACCCA GCAGATTGAG	3000
TATCTAGTTC ACAAGETTAG GCCCACACTG AAAGATGCAT GGGAGGACTG TGAGATCCTC	3060

CAGTCTCTGC TCCTAGGGGT GTTTGGTACT GGGATCGCAA GTGCTTCTCA ATTTTGTAGG	3120
AGCTGGCTCA ACCACCCTGA CATCATCGGG TATATAGTTA ATGGAGTTGG GGTGTCTGG	3180
CAATGCCATC GTGTTAATGT CACGTTTCATG GCGTGGAAATG AGTCCACCTA TTACCCTCCA	3240
GTAGATTACA ATGGGCGGAA GTACTTCCTG AATGATGAGG GAAGGTTACA AACAAACACC	3300
CCCGAGGCAA GGCCAGGGCT TAAGCGGGTC ATGTGTTTCG GCAGGTACTT CCTAGGGACA	3360
GTAGGGTCTG GGGTGAAACC GAGGAGGATT CCGTACAATA AGACCTCACA TGA CTACCAC	3420
CTGGAGGAGT TTGAGGCAAG TCTCAACATG ACCCCTCAGA CCAATATCGC CTCGGGTCAT	3480
GAGACAGACC CCATAAATCA TGCTACGGA ACGCAGGCTG ATCTCCTCC ATACACCAGG	3540
TCTAGTAATA TAACATCTAC GGATACAGGC TCAGGCTGGG TGACATCGG CCTACCCTCA	3600
TTTGCTTTCC TCAATCCCT CCGGTGGCTC AGGACCTAC TTGCATGGG AGCCTGGTTG	3660
GGTGGGGTTC TATACTTAAT AAGTCTTTGT GTTTCCTTAC CAGCCTCCTT CGCGAGGAGG	3720
AGACGCTCG GCCGGTGGA GGAATAAACC GTACCGACCA GTCTCTTAA AACCTCTCC	3780
TCGGAACAGA GGTCTCTTC TGCTTAAGT CGAGCTCACT CCCCATCAT GTACGAGCAC	3840
TAGGCCAGAT TAAAGCAAGG AACCTGGCAT CCTGTGACTA TTA CTGCTA TTCCGCCAAG	3900
TTGTATTGCC CCCTGAAGTA TATCCATTG GTGTTCTAAT AAGAGCTGG GAGGCTATAC	3960
TAACAGTTAT AGTATCAGCT TGAAGCTGG ATCATATGAC GAAGACCCTA TACTCCTCTG	4020
TGAGATATGC ACTCACCAAT CCCCAGGTC GAGCCCACT TGAGCTTAC ATTGCCTACC	4080
AGCCATAGT GGGTCAGGC TCCTACAGCC GGGAGGCGA CATAGGGCCA AAAAGGCTTG	4140
GGAAATATGTC ATTGCAATTC ATCCAATCTC TCGTTATTGC CACCATAGAC ACGACAAGCT	4200
GCCTAATGAC CTACAACCAC TTTCTTGCTG CAGCAGACAC AGCCAAGAGC AGATGCCATC	4260
TCCTAATCGC CTCAGTGGT CAGGGGGCCC TTTGGGAACA AGGGTCATTT CTGATCATA	4320
TAATCAACAT GATCGACATA ATTGACTCAA TCAACCTCCC CCATGATGAT TACTTCACAA	4380
TTATTAAGTC TATCTTTCCC TACTCCCAAG GGCTTGTAT GGGGAGGCAT AATGTATCAG	4440
TCTCCTCTGA TTTCCGCTCC GTATTGCCA TTCTGAAT ATGCCCCGAA CTAGACAGCT	4500
TACTAAAAA ACTGCTCCAA CTGACCCCG TTCTCCTCT CATGGTCTCT TCGGTGCAGA	4560
AGTCATGGTA CTTCCCTGAG ATCCGAATGG TCGACGGGC ACGGAGCAG CTCACAAGA	4620
TGCGTGCGA GCTGGAACG CCCCAAGCCC TGCTGTCTGA CGGCCATACC CTCCTGTCAA	4680
TATTTGCGGC AGAGTTTATC AAAGGCTATG TCTCAAAGAA TGCGAAGTG CCGCCCGTAC	4740
ACCTGCTCCC AGGCTGTGAC AAATCCATAA AAAATGCGAG AGAGCTGGGC CGCTGGAGCC	4800
CGGCATTGTA CCGACGATGG CAGCTCTTCG AGAAGGTTGT CATTCTAAGA ATTGCTGACC	4860
TAGATATGGA TCCCGACTTC AACGATATTG TTAGCGATAA GCGGATAATC AGCTCAAGAA	4920
GGGACTGGGT ATTCGAGTAC AATGCAGCGG CCTTTTGGA GAAATACGGT GAACGTTGG	4980
AGAGGCCTCC TGCCAGGTGG GGACCGTCAC GACTTGTGAA TGCTCTAATC GATGGACGCT	5040
TAGACAATAT CCCAGCCCTG CTAGAGCCAT TTTACAGGGG AGCGGTTGAG TTCGAGGATC	5100
GGTTGACTGT GCTCGTGCTT AAGGAGAAAG AGTTAAAGGT AAAGGGAAGG TTCTTCTCGA	5160
AGCAACATT GGCAATCAGG ATATATCAGG TTGTTGCTGA AGCTGCACCT AAGAATGAGG	5220
TTATGCCATA CCTAAGACA CACTCAATGA CCATGAGCTC AACGGCTCTA ACTCACCTTC	5280

TTAACCGGET ATCACAATCT ATCACTAAGG GTGACTCCTT TGTTATTAAC CTTGACTATA	5340
GTTCCTGGTG CAACGGTTTC CGACCAGAAC TGCAGGCCCC AATCTGTCGT CAGTTGGATC	5400
AGATGTTCAA TTGCGGGTAC TTCTTCAGGA CTGGGTGCAC ACTGCCATGC TTTACCACGT	5460
TTATTATTCA AGACAGGTTT AACCCGCCCT ATTCCTCAG TGGTGAGCCC GTTGAAGACG	5520
GAGTTACATG CGCGGTTGGG ACTAAACAA TGGGGGAGGG CATGAGGCAG AACTATGGA	5580
CAATCCTTAC GAGCTGCTGG GAGATAATTG CTCTTCGGGA AATTAACGTG ACGTTTAACA	5640
TACTAGGCCA AGGTGATAAT CAGACAATCA TCATACATAA ATCTGCAAGC CAAAATAACC	5700
AGCTATTAGC GGAGCGAGCA CTAGGGGCCC TGTACAAGCA TGCTAGATTA GCTGGCCATA	5760
ACCTCAAGGT AGAGGAATGC TGGGTGTCAG ATTGTCTGTA TGAGTATGGA AAGAAGCTTT	5820
TCTCCGTGG TGTACCTGTC CCGGGCTGTT TGAAGCAGCT CTCACGGGTG ACGGATTCTA	5880
CTGGAGAGCT ATTCCTAAC CTATACTCAA AGTTAGCCTG CTTAACATCA TCGTGTTTAA	5940
GCGCAGCGAT GGCAGACACA TCTCCATGGG TGGCACTCGC GACAGGTGTC TGTCTGTATC	6000
TTATCGAGTT ATATGTTGAG CTGCCTCCAG CAATCATGCA GGATGAGTCG CTATTGACGA	6060
CCCTCTGCCT CGTAGGCCCA TCCATTGGTG GGCTTCGAC CCCTGCAACC CTACCCAGTG	6120
TCTTTTTCAG AGGAATGTCC GACCCACTGC CCTTTCAGCT AGCACTCTTG CAGACCCTCA	6180
TTAAGACGAC AGGGGTGACC TGTAAGTTGG TGAATCGTGT GGTCAAGTTA CGGATAGCAC	6240
CCTATCCAGA CTGGCTCTCT CTAGTGACTG ACCCGACCTC ACTCAACATT GCCCAAGTGT	6300
ACCGGCCAGA ACGTCAGATC AGGAGGTGGA TTGAGGAAGC GATAGCGACA AGCTCACACT	6360
CGTCACGCAAT AGCAACTTTC TTCCAGCAGC CCCTCACGGA GATGGCTCAG TTGCTTGCGA	6420
GGGACCTTTC AACAAATGATG CCTCTTCGAC CCCGGGATAT GTCGGCCTTA TTCGCATTAT	6480
CAAAATGTCG ATACGGTTTA AGCATTATAG ATCTATTTCA AAAATCCTCT ACCGTTGTTT	6540
CTGCAAGTCA AGCTGTCCAT ATCGAGGATG TTGCCCTAGA GAGTGAAGG TATAAGGAAT	6600
CTATCATCCA GGGTCTGTTA GACACCACTG AGGGGTATAA CATGCAACCT TATTTGGAAG	6660
GTTGCACCTA CCTTGACGCC AACAGTTAC GTAGGTTGAC ATGGGGTCGA GACCTAGTTG	6720
GAGTCACAAT GCCGTTTGTG GCCGAGCAAT TCCATCCTCA CAGTTCTGTG GGTGCAAGG	6780
CGGAACCTTA CCTCGACGCT ATTATATACT GCCCAGGGA GACATTGCGG TCACACCATC	6840
TGACTACCAG GGGGGACCAG CCGCTTTACC TCGGATCCAA TACGGCTGTC AAGGTCCAGC	6900
GAGGTGAGAT CACGGGCCTA ACAAAGTCAA GGGGTGCAAA TCTAGTCAGG GACACTCTCG	6960
TTCTCCATCA GTGGTATAAA GTCCGTAAAG TTACCGATCC ACACTGAAC ACCCTCATGG	7020
CACGCTTCTT ACTTGAGAAG GGGTACACAT CTGACGCTCG ACCTAGCATC CAGGGTGGGA	7080
CCCTCACGCA TCGTCTCCCA TCCCGGGGAG ACTCACGGCA GGGGCTTACT GGGTATGTAA	7140
ATATACTAAG TACGTGGCTT CGATTCTCAA GTGATTATCT TCACTCTTTC TCGAAATCAT	7200
CAGACGACTA TACAATCCAC TTTCAGCATG TATTCACATA CGGTTGCCTC TATGCTGATT	7260
CGGTGATTAG ATCGGGCGGT GTTATTTCCA CTCCTTACCT TTTGAGTGCA AGTTGTAAAA	7320
CATGCTTTGA GAAGATAGAC TCAGAGGAGT TCGTCCCTGC ATGTGAACCC CAATACAGGG	7380
GTGCTGAGTG GCTGATATCA AAGCCAGTCA CTGTCCCTGA GCAGATAACT GATGCTGAAG	7440
TCGAGTTTGA CCCCTGTGTG AGTGCGGGTT ATTGTCTCGG GATTCTCATT GGCAAGTCAT	7500

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TCTTAGTTGA CATAAGGGCA AGTGGGCATG ATATCATGGA GCAGCGGACA TGGGCTAACC	7560
TGGAGAGGTT TTCTGTATCG GACATGCAGA AACTTCCGTG GAGTATTGTA ATTCCGTCTC	7620
TCTGGAGATT CCTTATTGGC GCACGGCTCC TTCAGTTTGA GAAGGCTGGC CTCATTAGAA	7680
TGCTGTATGC TGGACAGGT CCAACCCCTA GCTTCCTAAT GAAAGTTTTT CAAGACTCAG	7740
CCCTCCTCAT GGACTGCGCA CCCCTCGATC GGCTGTCCCC TAGGATCAAC TTTCATAGTC	7800
GGGGAGACCT CGTTGCTAAG CTGTGTTTAT TGCCCTTCAT CAACCCGGGT ATAGTGGAGA	7860
TTGAAGTGC TGGAAATTAAT AGCAAGTACC ATGCAGTATC GGAGGCCAAT ATGSATCTGT	7920
ACATCGCTGC TGCCAAGTCT GTGGCGTGA AGCCACACA GTTTGTTGAG GAAACAAACG	7980
ACTTTACGGC CCGCGGCCAC CACCATGGTT GTTATTCCTT TTCTTGGTCT AAGTCACGCA	8040
ATCAATCACA GGTCTTAAAG ATGGTAGTAC GGAAGCTGAA GCTCTGTGTC CTGTATATAT	8100
ACCCACAGT CGATCCCGCC GTTGCTCTCG ACCTGTGCCA TCTACCAGCA TTAATAATAA	8160
TCCTAGTGCT CGGCGGTGAC CCAGCGTACT ATGAGCGATT ACTTGAGATG GACCTGTGCG	8220
GGGCTGTGTC AAGTCGAGTC GATATCCCCC ATTCTCTGGC TGGCAGAACG CACAGGGGGT	8280
TCGAGTGGG CCCAGACGCT GGTCCAGGTG TAATTAGACT CGACAGGTTA GAGTCAGTTT	8340
GTTATGCTCA CCCCTGTTTA GAGGAAC TAGTTAATGC ATATCTAGAC TCTGAGTTGG	8400
TTGACATTAG TGATATGTGC TGCCCTCCCT TAGCGACACC CTGTAAGGCC CTTTTCAGGC	8460
CAATATATCG GAGCTTACAG TCGTTCAGGT TAGCCTTAAT GGACAACTAT AGTTTGTCA	8520
TGGACCTCAT TATGATCCGA GGACTGGACA TTAGGCCTCA CCTTGAGGAA TTTGACGAGC	8580
TGCTTGTGGT AGGACAGCAC ATCTCGGCC AGCCCGTCCT AGTAGAGGT GTTACTACG	8640
TTGAGTTGT TAGGAAGCGC CCTGTGTTAG CGAGGCATCC GTGGTCAGCA GATCTTAAGC	8700
GAATTACTGT GGGGGGGCGG GTCCTCTGCC CCTCTGCTGC CAGATTGCGT GATGAGGATT	8760
GTCAGGGGTC TCTGTTGGTT GGGCTTCTG CTGGTTGAC GCAGTTATTG ATAATTGATT	8820
AAGATCAAGC CACCTACTAC CCTATTCTTA AAAAACCATA TGTCAGTGGT SCAGTGCTTG	8880
GGCTTGGTTG TTGCTTTGTT GTAGCGCGTT	8910

## (21) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 8 amino acids  
 (B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met	Ala	Thr	Arg	Pro	Ser	Ser	Leu
1						5	

## (22) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 12 amino acids  
 (B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asn Ala Leu Thr Gln Pro Val Asp Gln Leu Leu Lys  
1 5

10

(23) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Asp Gln Pro Thr Gly Arg Glu Gln  
1 5

(24) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Val Arg Gly Thr Leu Gly Asp Ile  
1 5

(25) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Thr Ala Gln Arg Cys Asp His Ser  
1 5

(26) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO



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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Glu Thr Met Lys Leu Met Met Glu Lys Val Asp  
1 5

10

(27) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Pro Met Leu Pro Ser His Pro Ala  
1 5

(28) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Thr Ala Asp Glu Trp Asp Ile Ile  
1 5

(29) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Asn Ser Lys His Ser Tyr Val  
1 5

(30) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Thr Leu Met Leu Glu Ile Asp Phe  
1 5

## (31) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly His Ser Leu Val Asn Ile Tyr Phe Gln Ile Asp  
1 5

10

## (32) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Lys Asp Pro Ile Arg Lys Tyr  
1 5

## (33) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ala Phe Asn Val Phe Ser Tyr Arg  
1 5

## (34) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2658 amino acids  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GGTAGACCAG CTCCTGAAGG ACCTCAGGAA GAACCCCTCC ATGATCTCAG	50
ACCCAGACCA GCGAACCGGA AGGGAGCAGC TATCGAATGA TGAGCTTATC	100
AAGAAGCTAG TGACGGAGCT GGCCGAGAAT AGCATGATCG AGGCTGAGGA	150
GGTGCGGGGC ACTCTTGGGG ACATCTCGGC TCGCATCGAG GCAGGGTTTG	200
AGTCCCTGTC CGCCCTCCAA GTGGAAACCA TCCAGACAGC TCAGCGGTGC	250
GACCACTCCG ATAGCATCAG AATCCTTGGC GAGAACATCA AGATACTGGA	300
TCGCTCCATG AAGACAATGA TGGAGACAAT GAAGTCATG ATGGAGAAGG	350
TGGACCTCCT CTACGCATCA ACCGCCGTG GGACCTCTGC ACCCATGTTG	400
CCCTCCCATC CTGCACCTCC GCGCATTTAT CCCAGCTCC CAAGTGGCCC	450
GACAGCGGAT GAGTGGGACA TCATACCATA AAAAAATCGA ATCACCATGA	500
ATTCAAAGCA TTCCTATGTG GAGCTCAAGG ACAAGGTAAT CGTCCCTGGA	550
TGGCCACAC TGATGCTTGA GATAGACTTT GTAGGAGGGA CTTACGGAA	600
CCAGTTCCCT AACATCCCAT TTCTTTCAGT GAAAGAGCCT CTGCAGCTTC	650
CACGCGAGAA GAAGTTGACC GACTACTTCA CCATTGACGT AGAGCCAGCA	700
GGTCATTCCC TGGTCAACAT ATACTCCAG ATTGACGACT TCTTGCTCT	750
AACACTCAAC TCACTGTCCG TATACAAGGA CCCGATTAGG AAATACATGT	800
TCCTACGECT CAACAAGGAA CAGAGCAAGC ACGCAATTAA TGCAGCTTTC	850
AATGTCTTCT CTTATCGGCT TCGGAACATT GGTGTTGGCC CTCTCGGCCC	900
AGACATTGCA TCTTCAGGGC CTTAGTTGCA ATACTGACTC CACTCCTGGA	950
TTAATCGATC TGGAGATAAG GCGACTTTGC CACACCCCAA CGGAAAATGT	1000
CATTTTCATGC GAGGTTAGTT ATCTTAACCA CAGCACTATT AGCCTCCCGG	1050
CAGTCCACAC GTCATGCCTC AAGTACCACT GCAAAACCTA TTGGGGATTC	1100
TTTGGTAGCT ACAGCGCTGA CCGAATCATC AATCGGTACA CTGGTACTGT	1150
TAAGGGTTGT TTAACAACCT CAGCGCCAGA GGATCCCTTC GAGTGCAACT	1200
GGTTCTACTG CTGCTCGGCG ATTACAACAG AGATCTGCCG ATGCTCTATT	1250
ACAAATGTCA CGGTGGCTGT ACAGACATTC CCACCGTTCA TGTAAGCAG	1300
TTTCGCGGAC TGTAGTACTG TGAGTCAGCA GGAGCTAGAG AGTGGAAAGG	1350
CAATGCTGAG CGATGGCAGT ACCTTAACCT ATACCCCGTA TATCTTACAA	1400
TCAGAAGTCG TGAACAAAAC CCTTAATGGG ACTATACTCT GCAACTCATC	1450
CTCCAAGATA GTTTCCTTCG ATGAATTTAG GCGTTCATAC TCCCTAGCGA	1500
ATGGTAGTTA CCAGAGCTCA TCAATCAATG TGACGTGTGT AAATACACG	1550
TCGTCCTGCC GGTCCAAGTT GAGAAGGCGG CGTAGGGATA CTCAACAGAT	1600
TGAGTACCTA GTTCACAAGC TTAGGCCTAC ACTGAAAGAT GCCTGGGAGG	1650
ACTGTGAGAT CCTCCAGTCT CTGCTCCTAG GGGTGTGTTG TACTGGGATT	1700
GCAAGTGCTT CGCAATTCTT GAGGGGCTGG CTCAACCACC CTGATATCAT	1750
CGGGTATATA GTTAATGGAG TTGGGGTAGT CTGGCAATGC CATCGTGTG	1800

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ATGTCACGTT CATGGCGTGG AATGAGTCCA CATATTACCC TCCAGTAGAT	1850
TACAATGGAC GGAAGTACTT TCTGAATGAT GAGGGGAGGC TACAAACAAA	1900
CACCCCCGAG GCAAGGCCAG GGCTTAAGCG GGTCATGTGG TTCGGCAGGT	1950
ACTTCCTAGG GACAGTAGGG TCTGGGGTGA AACCGAGGAG GATTCGGTAC	2000
AATAAGACCT CACATGATTA CCATCTAGAG GAGTTTGAGG CAAGTCTCAA	2050
CATGACCCCC CAGACCAGTA TCGCCTCGGG TCATGAGACA GACCCCATAA	2100
ATCATGCCTA CGGAACGCAG GGTGACCTCC TTCCATACAC CAGGTCTAGT	2150
AATATAACGT CTACAGATAC AGGCTCAGGC TGGGTGCACA TCGGCCTACC	2200
CTCATTTGCT TTCCTCAATC CTCTCGGGTG GCTTAGGGAC CTACTTGCCT	2250
GGGCGGCCCTG GTTGGGTGGG GTTCTATACT TAATAAGTCT TTGTGTTTCC	2300
YTACCAGCCT CTTTCGCGAG GAGGAGACGC CTCGGCCGGT GGCAGGAATA	2350
AACCGTACCG ACCAATCTCT TAAAAACCCT CTTCTCGGGA CAGAGGTCTC	2400
TTTCTGCCCTT AAATCGAGTT CACTCCCCCA TCACGTACGA GCATTGGGCC	2450
AGATTAAAGC AAAGAACCTG GCATCCTGTG ACTATTACTT GCTATTCGCG	2500
CAAGTTGTAT TGCCCCCTGA AGTATATCCC ATTGGTGTCT TAATAAGAGC	2550
TGCGGAGGCC ATACTAACAG TTATAGTATC AGCTTGGGAG CTGGATCACA	2600
TGACAAAGAC CCTATACTCC TCTGTGAGAT ATGCACTCAC CAATCCCCGG	2650
GTCCGGGC	

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## (35) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGCAATCAAT GCAGC

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## (36) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 16 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (B) TYPE: amino acid

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TTCCTGCCAC CGGCCG

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We claim:

1. A nucleotide sequence selected from the group consisting of:

- a) SEQ ID NOs. 5, 7, 9, 19, 33; and their  
5 complementary nucleotide sequences;
- b) nucleotide sequences capable of hybridizing to SEQ ID NOs. 5, 7, 9, 19, 33 and their complementary nucleotide sequences, wherein said nucleotide sequence is at least 10 nucleotides in length  
10 and is not SEQ ID NOs. 1 or 3; and
- c) nucleotide sequence fragments selected from the group consisting of: fragments of SEQ ID NOs. 5, 7, and 9; nucleotide number 1 through 53, and nucleotide number 1880 through 8910 of SEQ ID NO 19;  
15 complementary nucleotide sequences of the foregoing; and nucleotide sequences capable of hybridizing to the foregoing; wherein each of said fragment encodes a protein comprising at least four amino acids or at least one immunogenic epitope.

- 20 2. An amino acid sequence selected from the group consisting of: SEQ ID NOs 6, 8, 10, 20 to 32; amino acid position 1 to amino acid position 102 of p23; amino acid position 37 to amino acid position 201 of p23; amino acid position 102 to amino acid position 201 of p23; amino  
25 acid position 154 to amino acid position 201 of p23; amino acid position 1 to amino acid position 70 of unglycosylated gp18; amino acid position 23 to amino acid position 142 of unglycosylated gp18; amino acid position 63 to amino acid position 142 of unglycosylated gp18;  
30 amino acid position 100 to amino acid position 142 of unglycosylated gp18; SEQ ID Nos. 20 to 32; a fragment of a full length amino acid sequence of p23, said fragment is selected from the group consisting of: amino acid position 1 to amino acid position 102 of p23, amino acid  
35 position 37 to amino acid position 201 of p23, amino acid

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position 102 to amino acid position 201 of p23, amino acid position 154 to amino acid position 201 of p23; a fragment of a full length amino acid sequence of gp18, said fragment is selected from the group consisting of:

5 amino acid position 1 to amino acid position 70 of gp18, amino acid position 23 to amino acid position 142 of gp18, amino acid position 63 to amino acid position 142 of gp18, amino acid position 100 to amino acid position 142 of gp18; fragments of the foregoing comprising at

10 least four amino acids or at least one immunogenic epitope; variants and equivalent amino acid sequences of the foregoing.

3. A substantially pure composition of protein gp18, p57, or pol.

15 4. A composition comprising: recp18, recp57, recpol, a recombinantly produced Borna disease virus, or a recombinantly produced virus encoded by the nucleotide sequence group consisting of: SEQ ID NOs. 5, 7, 9, 19, 33; and their complementary nucleotide sequences.

20 5. A recombinant cell, virus, or vector, containing a nucleotide sequence of claim 1, or a nucleotide sequence encoding the amino acid sequence of claims 2, 3, or 4.

25 6. An infective but replication deficient viral vector produced by removing or mutating at least one gene responsible for replication from a nucleotide sequence selected from the group consisting of SEQ ID No. 19, and a nucleotide sequence complementary or hybridizable to SEQ ID No. 19.

7. A method of delivering a desired gene or desired chemical into a host, comprising the step of administering into the host the viral vector of claim 6, or transplanting into the host a cell containing the viral vector of claim 6, said viral vector further comprising the desired gene, or a gene encoding the desired chemical.

8. A method for detecting, in a sample, a ligand capable of binding one or more proteins selected from the group consisting of: gp18, recp18, p57, recp57, pol, recpol, proteins having the amino acid sequences of claim 2, and immunogenic fragments thereof, said method comprising the steps of:

- a) contacting the sample with one or more of the proteins; and
- b) determining binding of the ligand to the proteins.

9. The method of claim 8, wherein the method is an immunoassay method, the ligand is an antibody immunoreactive with Borna disease virus, the proteins are prebound to a solid support without gel size fractionation.

10. A method for selecting for a ligand capable of binding one or more protein(s) selected from the group consisting of: gp18; recp18; p57; recp57; pol; recpol; proteins having the amino acid sequences of claim 2; and immunogenic fragments of the foregoing; said method comprising the steps of:

- a) contacting the ligand with the protein(s);
- b) selecting for the ligand which binds to the protein(s).

11. The method of claim 10, wherein the ligand is a therapeutic agent against a disease selected from the group consisting of: (1) BDV infection or related pathogenesis; and (2) neurologic and neuropsychiatric disease not due to BDV infection, said method further comprising the steps of:

a) treating patients suffering from the disease, with the selected ligand and assaying for efficacy of the selected ligand for combating the disease; and

b) selecting the ligand which is efficacious against the disease, said ligand serving as the therapeutic agent.

12. A ligand capable of binding a protein selected from the group consisting: gp18, p57, recp18, recp57, pol, recpol, proteins having the amino acid sequences of claim 2, and fragments thereof which contain at least one immunogenic epitope.

13. An assay panel comprising one or more proteins selected from the group selected from the group consisting of: gp18; recp18; p57; recp57; pol; recpol; proteins having the amino acid sequences of claim 2; and immunogenic fragments of the foregoing.

14. A method for detecting a nucleotide sequence in a sample comprising the steps of:

(a) contacting the sample to a test nucleotide sequence selected from the group consisting of: nucleotide sequences encoding genome of Borna disease virus, gp18, p57, and pol; fragments of genome of Borna disease virus which are at least 10 nucleotides in length and between nucleotide number 1 through 53, and nucleotide number 1880 through 8910 of SEQ ID NO 19; fragments of nucleotide sequences encoding gp18, p57, pol, which are at least 10 nucleotides in length; and



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nucleotide sequences which are complementary or hybridizable to any of the foregoing nucleotide sequences;

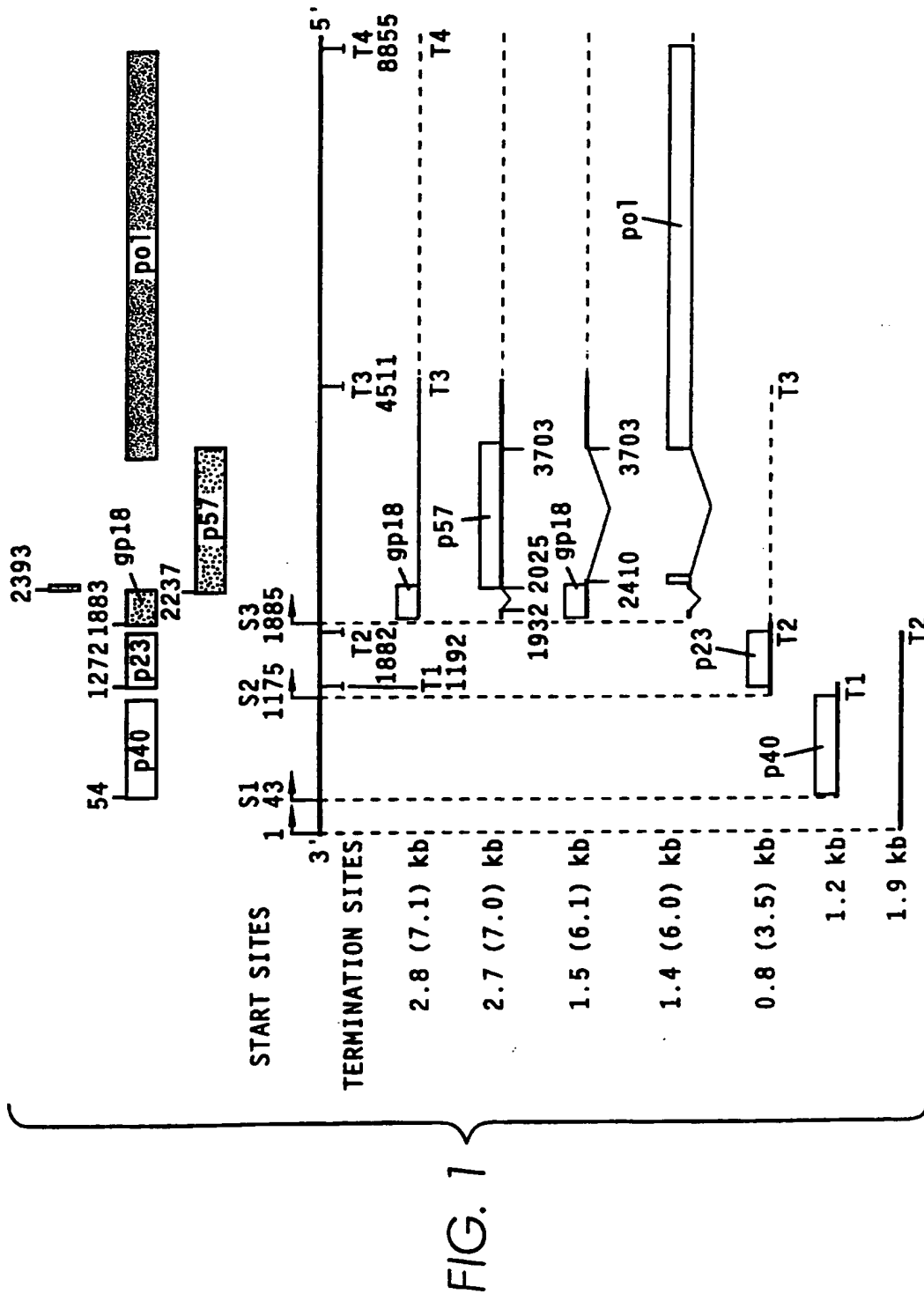
(b) incubating the sample with the test  
5 nucleotide sequence under hybridization condition; and

(c) detecting hybridization of any nucleotide sequence in the sample with the test nucleotide sequence.

15. A method for treating a patient suffering from:  
(1) BDV infection or related pathogenesis; or (2)  
10 neurologic and neuropsychiatric disease not due to BDV infection, said method comprising the steps of administering to the patient a therapeutic amount of the therapeutic agent of claim 11.

16. A vaccine comprising gp18, recp18, p57, recp57,  
15 pol, recpol, p23, recp23, p40, recp40, recombinantly produced Borna disease virus, the proteins of claim 2, fragments of the foregoing, or their encoding nucleotide sequences, capable of provoking cellular or humoral immune response in an organism administered with the  
20 vaccine.

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## FIG. 2(1)

1 GTTGGCTTAACAACAACCACTCATCTTCTTAACAAAATGAACACACGCAATGCCACCAAGAGACGCCTGTTGATGACGCCGATGCCATGGAGGA 100  
M P P K R R L V D D A D A H E D

101 TCAAGATCTATATGAACCCCAAGCAGCCTCCCTAAGCTCCCTGGGAAATTCCTACAATACACCGTTGGGGGGCTGACCCGATCCGGGTATAGGGCAT 200  
Q D L Y E P P A S L P K L P G K F L Q Y T V G G S D P H P G I G H

201 GAGAAGACATCAGGCAGAACGCACTGGCATTGTTAGACCACTCAGCGCGCATATGTTTACACAGTAACGCTAGCCTGTGTTTCTATGTTGCTAA 300  
E K D I R Q N A V A L L D Q S R R D M F H T V T P S L V F L C L L I

301 TCCCAGGACTGCACGCTGCTTTGTTACGAGGGGTGCTCTGTAATCTACCTGTGACGCTGTACGCTGGAGAACAGACTGTTGTTAAGACTGC 400  
P G L H A A F V H G G V P R E S Y L S T P V T R G E Q T V V K T A

401 GAAGTTTACGGGAAAAGACGACGAGCTGATCTACCGAGCTGGAGATCTCTCTATCTTCAGCCATTGTTGCTCATTACTAATAGGGTTGTGATA 500  
K F Y G E K T T Q R D L T E L E I S S I F S H C C S L L I G V V I

501 GGATCGTCTTAAGATCAAGCAGGAGCCGAGCAGATCAAGAAAAGGTTTAAACTATGATGGCAGCCTTAACCGGCCATCCATGGTGAGACTGCTA 600  
G S S S K I K A G A E Q I K K R F K T N H A A L N R P S H G E T A T

601 CACTACTCCAGATGTTTAAATCCACATGAGGCTATAGATTGGATTAAAGGCAACCTGGGTAGGCTCCTTTGTGTTGCTCTACTAACTACAGACTTTGA 700  
L L Q N F N P H E A I D W I N G Q P W V G S F V L S L L T T D F E

701 GTCCCCAGGTAAGAATTTATGGACCAGATTAAAGCTTGTGCGAAGTTATGCACAGATGACTACGTACTATAAAGGAGTACCTCGCAGATGCATG 800  
S P G K E F N D Q I K L V A S Y A Q M T T Y T T I K E Y L A E C H

801 GATGCTACCTTACAATCCCGTAGTTGCATATGAGATCCGTGACTTTTAAAGTTTCAGCAAGCTTAAGGAGGATCATGCTGACCTGTTCCCGTTTC 900  
D A T L T I P V V A Y E I R D F L E V S A K L K E D H A D L F P F L

901 TGGGGCCATTAGACACCCGACGCTATCAAGCTGGCGCCACGAAGCTTCCCAATCTGGCCTCCGAGCGTTTACTGGAGTAAGAAGGAAAACCCAC 1000  
G A I R H P D A I K L A P R S F P N L A S A A F Y W S K K E N P T

1001 AATGGCAGGCTACCGGGCTCCACCATCCAGCCGGGCGCAAGTGTCAAGGAACCCAGCTTGCCCGGTATAGGCGCCGCGAGATATCTGTTGAGAGGAC 1100  
H A G Y R A S T I Q P G A S V K E T Q L A R Y R R R E I S R G E D

1101 GGGGAGAGCTCTCAGTGAGATCTGCCATAATGAAGATGATAGGTGACTGGTCTAAACTAAAAACAATGAACAAACCAATAAAAAACCAATGC 1200  
G A E L S G E I S A I M K N I G V T G L N \*

1201 GGCAAACCTCCGCGACCTGCGATGAGCTCCGACCTCCGGCTGACATTGCTTGAATAGTCAGGAGGCTCAATGGCAACGCGACCATCGAGTCTGGTCGA 1300  
M A T R P S S L V D

1301 CTCCCTGGAGGACGAAGAAGATCCCAGACACTACGACGGGAACGACCGGGGTACCAAGACCACGGAAGGTCCCAAGGAATGCATTGACCCCAACAGTA 1400  
S L E D E E D P Q T L R R E R P G S P R P R K V P R N A L T Q P V

1401 GACCAGCTCTGAAGGACCTCAGGAAGAACCCTCCATGATCTCAGACCCAGACGACGAAACCGGAAGGAGCAGCTGTGAAATGATGAGCTAATCAAGA 1500  
D Q L L K D L R K N P S H I S D P D O R T G R E Q L S N D E L I K K

1501 AGTTAGTGACGAGCTGGCCGAGAATAGCATGATCGAGGCTGAGGAGGTGCGGGCACTCTTGGAGACATCTCGGCTCGTATCGAGGAGGGTTGAGTC 1600  
L V T E L A E N S H I E A E E V R G T L G D I S A R I E A G F E S

1601 CCTGTCCGCTCCAAAGTGAACCATCCAGACAGCTCAGCGGTGCGATCACTCCGACAGCATCAGGATCTCGGCGAGAACATCAAGATACTAGATCGC 1700  
L S A L Q V E T I Q T A O R C D H S D S I R I L G E N I K I L D R

1701 TCCATGAAGCAATGATGGAGCAATGAAGCTCATGATGGAGAAGTGGATCTCTCTACGATCAACCGCGCTTGGGACCTCTGCAACCATGTTGCCCT 1800  
S H K T M H E T M K L M H E K V D L L Y A S T A V G T S A P H L P S

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## FIG. 2(2)

1801 CCCATCCTGCACCTCCGCGATTTATCCCACTCCCAAGTGCCCGACACGGATGAATGGGACATCATACCATAAAAAATCGAATCACCATGAATTC 1900  
H P A P P R I Y P Q L P S A P T T D E W D I I P \* H N S

1901 AAAACATTCTATGTGGAGCTCAAGGACAAGGTAAATCGTCCCTGGATGGCCACACTGATGCTTGAGATAGACTTTGTAGGGGGGACTTCACGGAACAG 2000  
K H S Y V E L K D K V I V P G W P T L H L E I D F V G G T S R N Q

2001 TTCCTTAACATCCCATTTCTTTCAGTGAAGAGCCTCTGACGCTCCACGCGAGAAGAAGTTGACCGACTACTTTACTATTGACGTAGAACCAGCAGGTC 2100  
F L N I P F L S V K E P L Q L P R E K K L T D Y F T I D V E P A G H

2101 ATCCCTGGTCAATATATACTTCAGATTGACGACTTCTGCTCCTAACACTCAACTCACTATCTGTGTACAAGGACCCGATTAGAAAAATACATGTTCTT 2200  
S L V N I Y F O I D D F L L L T L N S L S V Y K D P I R K Y M F L

2201 ACGCCTCAAGGACAGAGCAAGCAGCAATCAATGACGCTTCAATGCTTTTCTTATCGGCTTCGGAACATTGCTGTTGCTCCTCTCGGCCCGGAC 2300  
R L R K D Q S K H A I N A A F N V F S Y R L R H I G V G P L G P D  
H Q P S H S F L I G F G T L V L V L S A R T

2301 ATTCGATCTTCAGGGCTTAGCTGCAATCTGACTCCACTCTGGAAGTATTGACCTGAGATAAGGCGACTTTGCCACACCCCAACGGAATATGTCATT 2400  
I R S S G P \*  
F D L Q G L S C H T D S T P G L I D L E I R R L C H T P T E N V I

2401 TCATGCGAGTTAGTTATCTCAACCACAGACTATTAGCTCCCGGAGTCCACACATCATGCTCAAGTACCAGTGCAAAACCTATTGGGGATTCTTTG 2500  
S C E V S Y L R H T T I S L P A V H T S C L K Y H C K T Y W G F F G

2501 GTAGCTACAGCGCTGACCGAATCATAATCGGTACACTGCTACTGTTAAGGGTTGCTAAACAACCTCAGCACCAGAGGACCCCTTCGAGTGAACCTGGT 2600  
S Y S A D R I I N R Y T G T V K G C L N H S A P E D P F E C H W F

2601 CTACTGCTGCTGGCGATTACAACAGAGATCTGCCGATGCTCTATTACAATGTACGGTGGCTGTGCAACATTCCCAACGCTCATGTACTGCAAGTTT 2700  
Y C C S A I T T E I C R C S I T N V T V A V Q T F P P F M Y C S F

2701 GCAGAGTGAAGTACCCTGAGCAACAGAGCTAGAGAGTGGAAAGGCAATGCTGAGCGATGGAGTACATTAACCTATATCCCGCTATATCTACAGTCA 2800  
A D C S T V S Q Q E L E S G K A H L S D G S T L T Y T P Y I L O S E

2801 AAGTCGTGAACAAACCTCAATGGGACCACTCTGCAACTCATCTCTAAGATAGTTTCTTCGATGAATTAAGGCTTCATACTCCCTAACGAATGG 2900  
V V N K T L N G T I L C N S S S K I V S F D E F R R S Y S L T N G

2901 TAGTTACAGAGCTCATCAATCAATGTGAGCTGTGCAAACTACAGCTGCTCTGCGGCCAGGTTGAAAGGCGGCTAGGGACACCCAGCAGATTGAG 3000  
S Y Q S S S I N V T C A N Y T S S C R P R L K R R R R D T Q Q I E

3001 TATCTAGTTCACAAGCTTAGGCCCACTGAAAGATGATGGGAGGACTGTGAGATCCTCCAGTCTCTGCTCTAGGGGTGTTTGTACTGGGATCGCAA 3100  
Y L V H K L R P T L K D A M E D C E I L Q S L L L G V F G T G I A S

3101 GTGCTTCTCAATTTTGAAGAGCTGGCTCAACACCTGACATCATCGGGTATAGTTAATGGAGTTGGGGTTGCTGGAATGCCATGCTGTTAATGT 3200  
A S Q F L R S V L N H P D I I G Y I V N G V G V V W Q C H R V N V

3201 CAGCTTCATGGCTGGAATGAGTCCACTATTACCTCCAGTAGATTACAATGGGCGAAGTACTTCTGAATGATGAGGGAAGGTTACAACAACACC 3300  
T F H A W N E S T Y Y P P V D Y N G R K Y F L N D E G R L Q T N T

3301 CCCGAGGCAAGGCCAGGGCTTAAGCGGCTCATGTGTTTGGCAGGACTTCTTAGGGACAGTAGGGTCTGGGGTGAACCGAGGAGGATTGGGTACAATA 3400  
P E A R P G L K R V N W F G R Y F L G T V G S G V K P R R I R Y N K

3401 AGACCTCACATGACTACCCTGGAGAGTTTGAAGGCAAGTCTCAACATGACCCCTCAGACCAATGATGCTCCTGGGTCATGAGACAGACCCATAAATCA 3500  
T S H D Y H L E E F E A S L N M T P Q T S I A S G H E T D P I N H

3501 TGCTACGGACGAGGCTGATCTCTTCCATACACCAAGTCTAGTAATATAACATCTACGGATACAGGCTCAGGCTGGGTGCACATCGGCTACCCCTCA 3600  
A Y G T Q A D L L P Y T R S S N I T S T D T G S G N V H I G L P S

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## FIG. 2(3)

3601 TTTGCTTTCTCAATCCCCCGGGTGGCTCAGGGACCTACTTGCATGGGAGCCTGGTTGGGTGGGTTCTATACTTAATAAGTCTTTGTTTCTTAC 3700  
F A F L N P L G W L R D L L A W A A W L G G V L Y L I S L C V S L P

3701 CAGCCTCTTTCGCGAGGAGAGAGCCTCGGCCGTGGCAGGAATAAACCGTACCGACCAGTCTCTTAAACCCTCTCCTCGGAACAGAGGTCTTTTC 3800  
A S F A R R R R L G R W Q E \*

3801 TGCCTAAGTCGAGCTCACTCCCCCATCATGTACGAGCACTAGGCCAGATTAAAGCAAGGAACCTGGCATCTGTGACTATTACTTGTATTCCGCCAAG 3900

3901 TTGTATTGCCCCCTGAAGTATATCCCATTTGGTGTCTAATAAGAGCTGCGGAGGCTATACTAACAGTTATAGTATCAGCTTGGAGCTGGATCATATGAC 4000  
H T

4001 GAAGACCTTACTCTCTGTGAGATATGCACTACCAATCCCCGGGTCCGAGCCCACTTGAGCTTCACATTGCCACAGCGCATAGTGGGTGAGGTC 4100  
K T L Y S S V R Y A L T N P R V R A Q L E L H I A Y Q R I V G Q V

4101 TCGTACAGCGGGAGGAGACATAGGGCCAAAAGGCTTGGGAATATGTATTGCAATTCATCAATCTCTCGTTATTGCCACCATAGACGACAGGCT 4200  
S Y S R E A D I G P K R L G N H S L Q F I Q S L V I A T I D T T S C

4201 GCCTAATGACCTACAACTTTCTTGTCTGACGAGACACAGCCAGAGAGATGCCATCTCCTAATCGCTCAGTGGTCCAGGGGGCCCTTTGGGAACA 4300  
L H T Y N H F L A A A D T A K S R C H L L I A S V V Q G A L W E Q

4301 AGGGTCATTCTTGATCATATAATCAACATGATCGACATAATTGACTCAATCAACCTCCCCCATGATGATTACTTCACAATTATTAAGTCTATCTTCCC 4400  
G S F L D H I I N H I D I I D S I N L P H D D Y F T I I K S I F P

4401 TACTCCCAAGGGCTTGTATTGGGGAGGCAATATGATCAGTCTCCTCTGATTTCGGTCCGTATTTGCCATTCTGAATTATGCCGCACTAGACAGCT 4500  
Y S Q G L V H G R H N V S V S S D F A S V F A I P E L C P Q L D S L

4501 TACTAAAAAAGTCTCCAACTTGACCCGTTCTCCTCTCATGGTCTCTCGGTGAGAGTCATGGTACTTCCCTGAGATCCGAATGGTGCAGGGGTC 4600  
L K K L L Q L D P V L L L H V S S V Q K S W Y F P E I R H V D G S

4601 ACGGGAGAGCTCCACAAGATGGGTGTGAGCTGGAACGCCCAAGCCCTGCTGTCTACGGCCATACCTCTCTGCAATATTTCGGGAGAGTTTATC 4700  
R E Q L H K H R V E L E T P Q A L L S Y G H T L L S I F R A E F I

4701 AAAGGCTATGTCTCAAGAAATGCGAAGTGGCCCGTACACCTGCTCCAGGCTGTGACAAATCCATAAAAAATGCGAGAGAGCTGGGCGCTGGAGCC 4800  
K G Y V S K N A K W P P V H L L P G C D K S I K N A R E L G R W S P

4801 CGGCATTGACCGACGATGGCAGCTCTTCGAGAGGTTGTCTTAAAGATTGCTGACCTAGATATGGATCCCGACTTCAACGATATTGTTAGCGATAA 4900  
A F D R R W Q L F E K V V I L R I A D L D H D P D F N D I V S D K

4901 GCGGATAATCAGCTCAAGAGGGAGTGGGTATTGAGTACAATGCAGCGGCTTTTGGAGAAATACGGTGAACGGTTGGAGAGGCTCTCTGCCAGGTCG 5000  
A I I S S R R D W V F E Y N A A A F W K K Y G E R L E R P P A R S

5001 GGACCGTCACGACTTGTGAATGCTCTAATCGATGGACGCTTAGACAATATCCAGCCCTGCTAGAGCCATTTACAGGGGAGCGGTTGAGTTGAGGATC 5100  
G P S R L V N A L I D G R L D N I P A L L E P F Y R G A V E F E D R

5101 GGTGACTGTGCTGCTAAGGAGAAAGAGTTAAAGGTAAGGGAAGGTTCTTCTGAAAGCAACATTGGCAATCAGGATATATCAGGTTGTGCTGA 5200  
L T V L V P K E K E L K V K G R F F S K Q T L A I R I Y Q V V A E

5201 AGCTGCACTTAAGAATGAGGTTATGCCATACCTAAAGACACACTCAATGACCATGAGCTCAACGGCTCTAACTCACCTTCTTAACCGGCTATCACATACT 5300  
A A L K N E V H P Y L K T H S H T H S S T A L T H L L N R L S H T

5301 ATCACTAAGGGTACTCTTGTATTAACTTGAATATGTTCTGCTGCAACGGTTTCGACGAGAACTGCAGGGCCCAATCTGTCTCAGTTGGATC 5400  
I T K G D S F V I N L D Y S S W C N G F R P E L Q A P I C R Q L D G

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## FIG. 2(4)

5401 AGATGTTCAATTGCGGGTACTTCTTCAGGACTGGGTGCACACTGCCATGCTTTACCAGTTTATTATTCAAGACAGGTTCAACCCGCCCTATTCCCTCAG 5500  
M F N C G Y F F R T G C T L P C F T T F I I Q D R F N P P Y S L S

5501 TGGTGAGCCGTTGAAGACGAGTTACATGCGGTTGGGACTAAACAATGGGGAGGGCATGAGGCAGAACTATGGACAATCCTTACGAGCTGCTGG 5600  
G E P V E D G V T C A V G T K T H G E G H R Q K L W T I L T S C W

5601 GAGATAATTGCTCTTGGGAAATTAACGTGACGTTTAACTACTAGGCCAAGGTGATAATCAGACAATCATCATATAAATCTGCAAGCCAAAATAACC 5700  
E I I A L R E I N V T F N I L G Q G D N O T I I I H K S A S Q N N O

5701 AGCTATTAGCGGAGCAGCACTAGGGCCCTGTACAAGCATGCTAGATTAGCTGGCCATAACCTCAAGGTAGAGGAATGCTGGGTGTCAAGTGTCTGTAT 5800  
L L A E R A L G A L Y K H A R L A G H N L K V E E C W V S D C L Y

5801 TGAGTATGAAAGAGCTTTTCTCCGTGTGCTGTCTCCGGGCTGTTTGAAGCAGCTCTCAGGGGTGACGGATTCTACTGGAGAGCTATTCCCAAC 5900  
E Y G K K L F F R G V P V P G C L K Q L S R V T D S T G E L F P N

5901 CTATACTCAAAGTTAGCCTGCTTAACATCATCGTGTAAAGCGCAGCATGGCAGACACATCTCCATGGGTGGCACTCGGACAGGTGTCTGTCTGTATC 6000  
L Y S K L A C L T S S C L S A A N A D T S P W V A L A T G V C L Y L

6001 TTATCAGTTATATGTTGAGCTGCCCAAGCAATCATGAGGATGAGTGGCTATTGACGACCCTCTGCTGAGGCCCATCCATTGGTGGGCTTCCGAC 6100  
I E L Y V E L P P A I N Q D E S L L T T L C L V G P S I G G L P T

6101 CCCTGCAACCTACCCAGTGTCTTTTCAAGGAATGTCGACCCACTGCCCTTTCAGCTAGCACTCTTGACAGCCCTCATTAAAGCAGAGGGGTGACC 6200  
P A T L P S V F F R G H S D P L P F Q L A L L Q T L I K T T G V T

6201 TGAGCTTGGTGAATGCTGTGGTCAAGTTACGGATAGCACTCTATCCAGCTGGCTCTCTAGTGACTGACCCGACCTCACTCAACATTGCCAAGTGT 6300  
C S L V N R V V K L R I A P Y P D W L S L V T D P T S L H I A Q V Y

6301 ACCGGCCAGAACGTCAGATCAGGAGGTGGATTGAGGAAGCATAGCGACAAGCTCACACTCGTCACGCATAGCAACTTTCTTCCAGAGCCCTCACGGA 6400  
R P E R Q I R R W I E E A I A T S S H S S R I A T F F Q Q P L T E

6401 GATGGCTCAGTTGCTTGGCAGGGACCTTTCAACAATGATGCTCTTGCAGCCCGGATATGTGGGCTTATTGCAATTATCAATGTCCGATACGGTTTA 6500  
H A Q L L A R D L S T H M P L R P R D M S A L F A L S N V A Y G L

6501 AGCATTATAGATCTATTTCAAAATCCTCTACCGTTGTTCTGCAAGTCAAGCTGTCCATATCAGGATGTTGCCCTAGAGAGTGAAGGTATAAGGAAT 6600  
S I I D L F Q K S S T V V S A S Q A V H I E D V A L E S V R Y K E S

6601 CTATCATCCAGGGTCTGTTAGACACCACTGAGGGGTATAACATGCAACCTTATTGGAAGGTTGCACCTTACCTTGACGCCAAACAGTTACGTAGGTTGAC 6700  
I I Q G L L D T T E G Y N M Q P Y L E G C T Y L A A K Q L R R L T

6701 ATGGGTCGAGACCTAGTTGAGTCACAATGCCGTTTGTGCGGAGCAATTCATCCTCAGTTCTGTGGGTGCAAGGCGGAACTCTACCTCGACGCT 6800  
W G R D L V G V T H P F V A E Q F H P H S S V G A K A E L Y L D A

6801 ATTATATACTGCCACAGGAGACATTGCGGTACACCATCTGACTACAGGGGGACCAAGCGCTTACCTCGGATCCAATACGGCTGTCAAGGTCCAGC 6900  
I I Y C P Q E T L R S H H L T T R G D Q P L Y L G S H T A V K V Q R

6901 GAGGTGAGATCAGGGGCTAACAAAGTCAAGGGGTGCAATCTAGTCAGGACACTCTGTTCTCCATCAGTGTATAAAGTCCGTAAAGTTACCGATCC 7000  
G E I T G L T K S R A A N L V R D T L V L H Q W Y K V R K V T D P

7001 ACACTTGAACCCCTCATGCGCGCTTCTACTTGAGAAGGGGTACACATCTGACGCTGACCTAGCATCCAGGGTGGGACCTCACGCATCTGTCCCA 7100  
H L N T L M A R F L L E K G Y T S D A R P S I Q G G T L T H R L P

7101 TCCCGGAGACTCAGGCGAGGGGCTTACTGGGTATGTAATATAAGTACGTGGCTTCGATTCTCAAGTATTATCTTCACTCTTCTCGAATCAT 7200  
S R G D S R Q G L T G Y V N I L S T W L R F S S D Y L H S F S K S S

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## FIG. 2(5)

7201 CAGACGACTATACAATCCACTTTTCAGCATGTATTACATACGGTTGCTCTATGCTGATTGCGTGATTAGATCGGGCGGTGTTATTTCCACTCCTTACCT 7300  
D D Y T I H F O H V F T Y G C L Y A D S V I R S G G V I S T P Y L

7301 TTTGAGTGCAAGTTGTAAACATGCTTTGAGAAGATAGACTCAGAGGAGTTGCTGCTGCGATGTGAACCCCAATACAGGGGTGCTGAGTGGCTGATATCA 7400  
L S A S C K T C F E K I D S E E F V L A C E P Q Y R G A E W L I S

7401 AAGCCAGTCAGTGTCCCTGAGCAGATAACTGATGCTGAAGTCGAGTTTGACCCCTGTGTGAGTGGGGTTATTGCTCGGGATTCTCATTGGCAAGTCAT 7500  
K P V T V P E Q I T D A E V E F D P C V S A G Y C L G I L I G K S F

7501 TCTTAAAGTACATAAGGGCAAGTGGGATGATATCATGAGCAGCGGACATGGGCTAACCTGGAGAGGTTTCTGTATCGGACATGCAAACTTCCGTG 7600  
L V D I R A S G H D I M E Q R T W A H L E R F S V S D M Q K L P W

7601 GAGTATTGTAATTGCGTCTCTGAGGATTCTTATTGGCGCAGGCTCTTCAAGTTTGAGAAGGCTGGCTCATTAGAATGCTGTATGCTGCGACAGGT 7700  
S I V I R S L W R F L I G A R L L Q F E K A G L I R M L Y A A T G

7701 CCAACCCCTAGCTCTTAATGAAAGTTTTCAAGACTCAGCCCTCTCATGGACTGCGCACCCTCGATCGGCTGTCCCTAGGATCAACTTTTCAAGTC 7800  
P T P S F L M K V F Q D S A L L M D C A P L D R L S P R I N F H S R

7801 GGGGAGAGCTCGTGTAAAGCTTTTATTGCCCTTCAATCAACCCGGGTATAGTGGAGATTGAAGTGTCTGGAATTAATAGCAAGTACCATGCAATATC 7900  
G D L V A K L V L L P F I N P G I V E I E V S G I N S K Y H A V S

7901 GGAGGCCAATATGGATGCTGTACATGCTGCTGCAAGTCTGTGGGCGTGAAGCCGACAGTTTGTGAGGAACAAACGACTTTACGGCCCGGCGCCAC 8000  
E A N M D L Y I A A A K S V G V K P T Q F V E E T N D F T A R G H

8001 CACCATGTTGTTATTCCTTTCTTGTCTAAGTCACGCAATCAATCAGAGTCTAAAGATGGTAGTACGGAAGCTGAAGCTCTGTCTGTATATAT 8100  
H H G C Y S L S M S K S R M Q S Q V L K M V V R K L K L C V L Y I Y

8101 ACCCCAGATCGATCCCGGCTTGTCTGCGACTGTGCCATCTACGAGTAACTATAATCTAGTGTCTGGCGGTGACCCAGCTACTATGAGCGATT 8200  
P T V D P A V A L D L C H L P A L T I I L V L G G D P A Y Y E R L

8201 ACTTGAGATGGACCTGTGCGGGCTGTGTCAAGTCGAGTGSATATCCCCATTCTCTGGCTGGCAGAACGACAGGGGTTCTGAGTGGGCCAGAGCT 8300  
L E M D L C G A V S S R V D I P H S L A G R T H R G F A V G P D A

8301 GGTCCAGGTGTAATAGACTCAGAGGTAGAGTCAGTTGTTATGCTCACCCTGTTTAGAGGAAGTGAAGTTAATGCATATCTAGACTCTGAGTTGG 8400  
G P G V I R L D R L E S V C Y A H P C L E E L E F N A Y L D S E L V

8401 TTGACATTAGTATGATGTGCTGCTCCCTTAgCGACACCCTGTAAggCCCTTTTCAGGCCAATATATCGGAGCTTACAGTCTTCAGGTTAGCCTTAAT 8500  
D I S D M C C L P L A T P C K A L F R P I Y R S L Q S F R L A L M

8501 GGACAATATAGTTTTGTGATGACCTCATTATGATCCGAGGACTGGACATTAGGCTCACCCTTGAGGAATTTGAGGAGCTGCTTGTGTTAGGACAGCAC 8600  
D H Y S F V M D L I H I R G L D I R P H L E E F D E L L V V G Q H

8601 ATCTCGGCCAGCCGCTCTAGTAGAGGTGTTTACTAGCTTGAAGTTGTTAGGAAGCCCTGTGTTAGCGAGGATCCGTGCTCAGCAGATCTTAAGC 8700  
I L G Q P V L V E V V Y Y V G V V R K R P V L A R H P W S A D L K R

8701 GAATTAAGTGGGGGGGGGCTCCCTGCTGCTGCGAGATTGCTGATGAGGATTGTGAGGGGCTCTGTTGGTTGGGCTTCTGCTGGGTTGAC 8800  
I T V G G R A P C P S A A R L R D E D C Q G S L L V G L P A G L T

8801 GCAGTTATTGATAATTGATTAAAGATCAAGCCACTACTACCTATTCTTAAAAACCATATGTCAAGTGGTCAAGTGTGAGGCTTGGCTTGGTTGTTGTT 8900  
Q L L I I D \*

8901 GTAGCGCGTT

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FIG. 3a

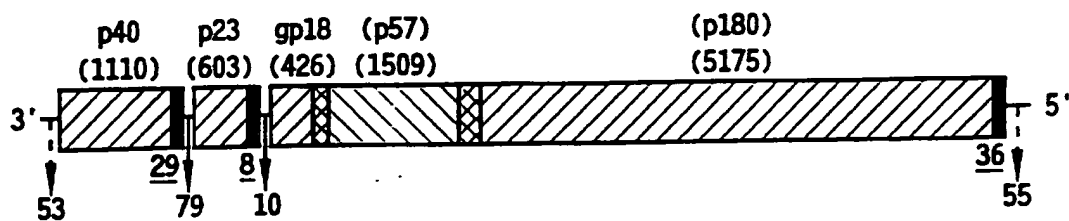


FIG. 3b

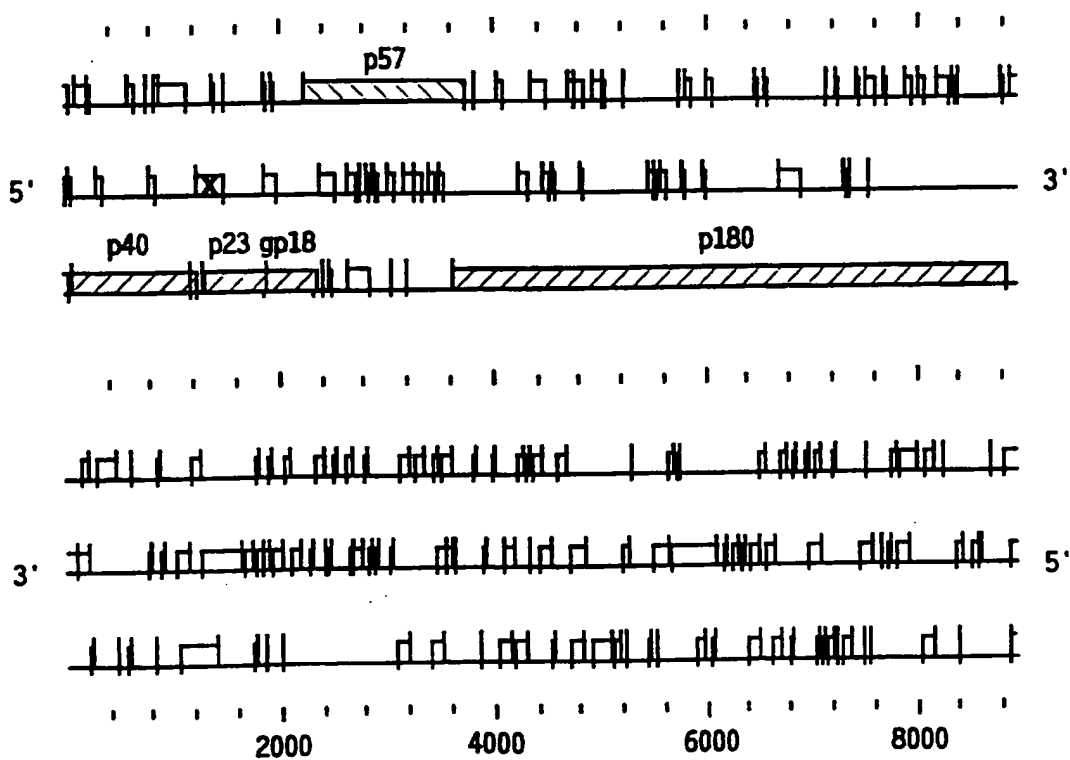




FIG. 4(1)

[illegible]

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FIG. 6a

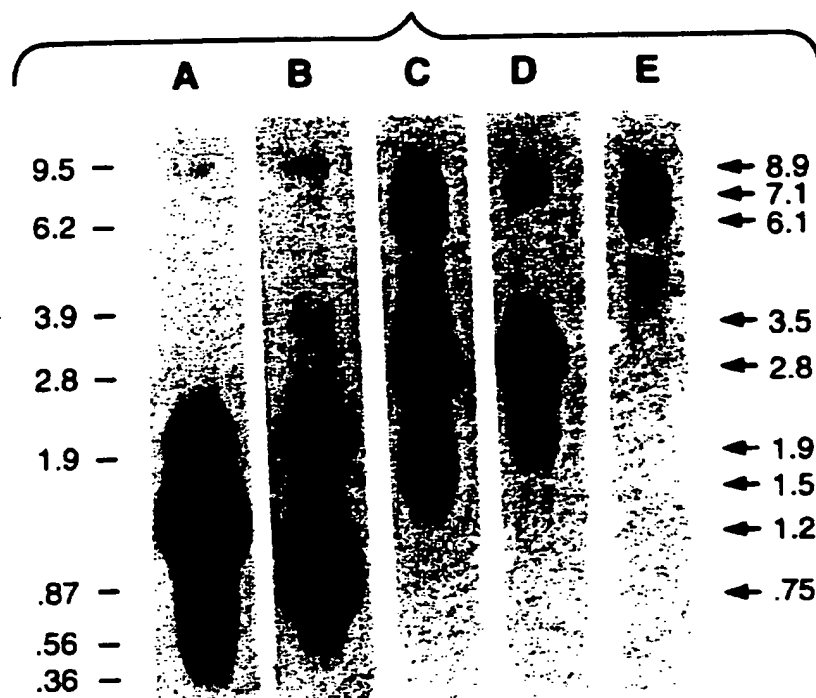
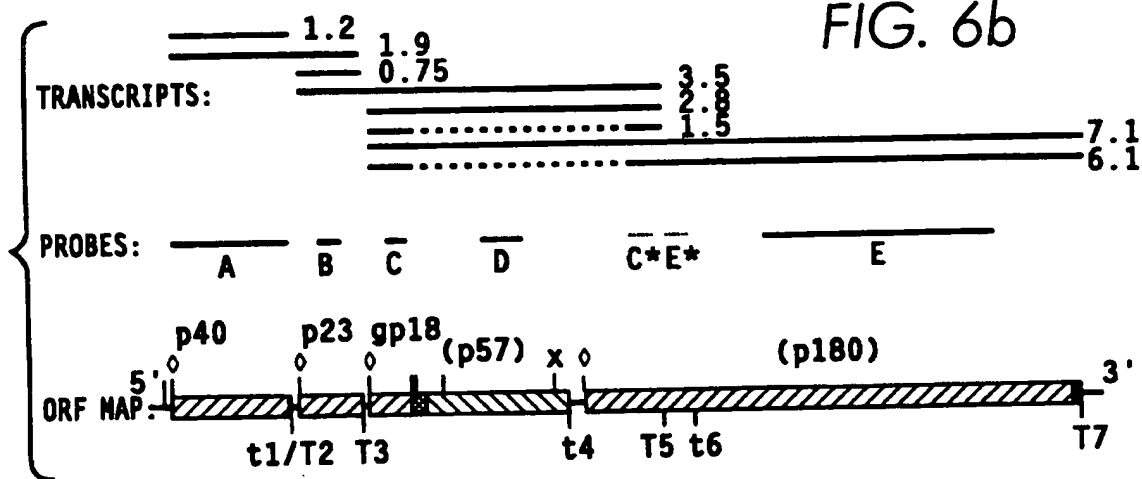


FIG. 6b



p40 5'-GUcU AAa cua <sup>(t1)</sup> aaC AAU GAA caa acc aaU aaaaaa CCA AAU GCG (T2)

P23 5'-CGAC AAC GGA UGA AUG GGA CAU cau acc aua aaaaaa UCG AAU CAC (T3)

gp18 5'-UUAG CUG CAA UAC UGA CUC CAC UCC UGG ACU GAU UGA CCU

P57 5'-GGCA GGA AUA AAC CGU ACC GAC cag Ucu cuu aaaaa CCC UCU CCU (t4)

5'-CUGA AUU AUG CCC GCA ACU AGA cag cuu acu aaaaaaACUG CUC CAA (T5)

5'-UACA CCU GCU CCC AGG CUG UGA caa auc cau aaaaaa UGC GAG AGA (t6)

P180 5'-UUAA GAU CAA GCC ACC UAC UAC ccu auu cuu aaaaa CCA UAU GUC (T7)

FIG. 6c



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FIG. 8

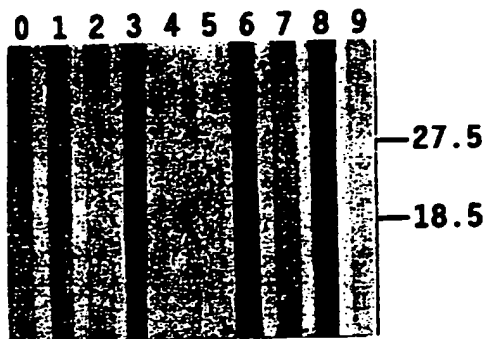


FIG. 9

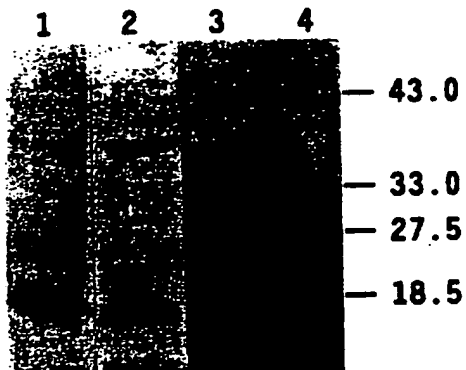


FIG. 10A

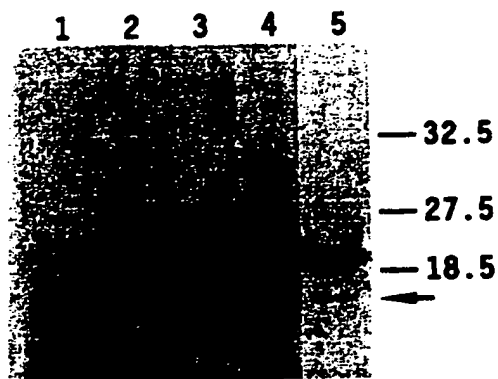


FIG. 10B

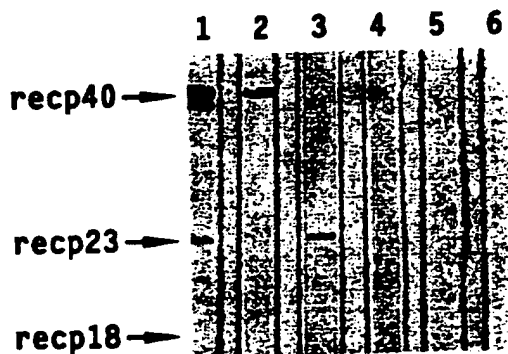
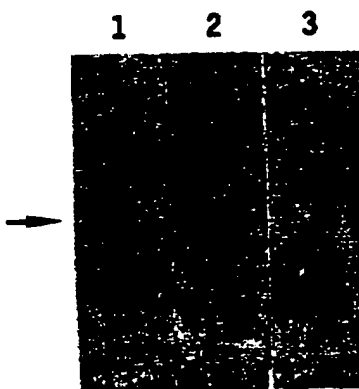


FIG. 11A

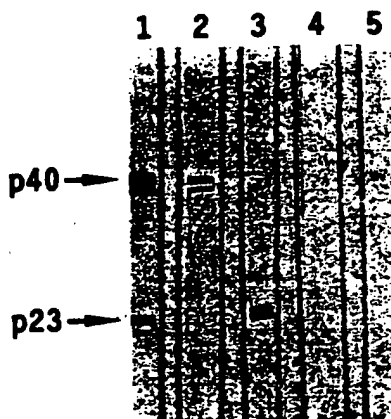


FIG. 11B

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FIG. 12

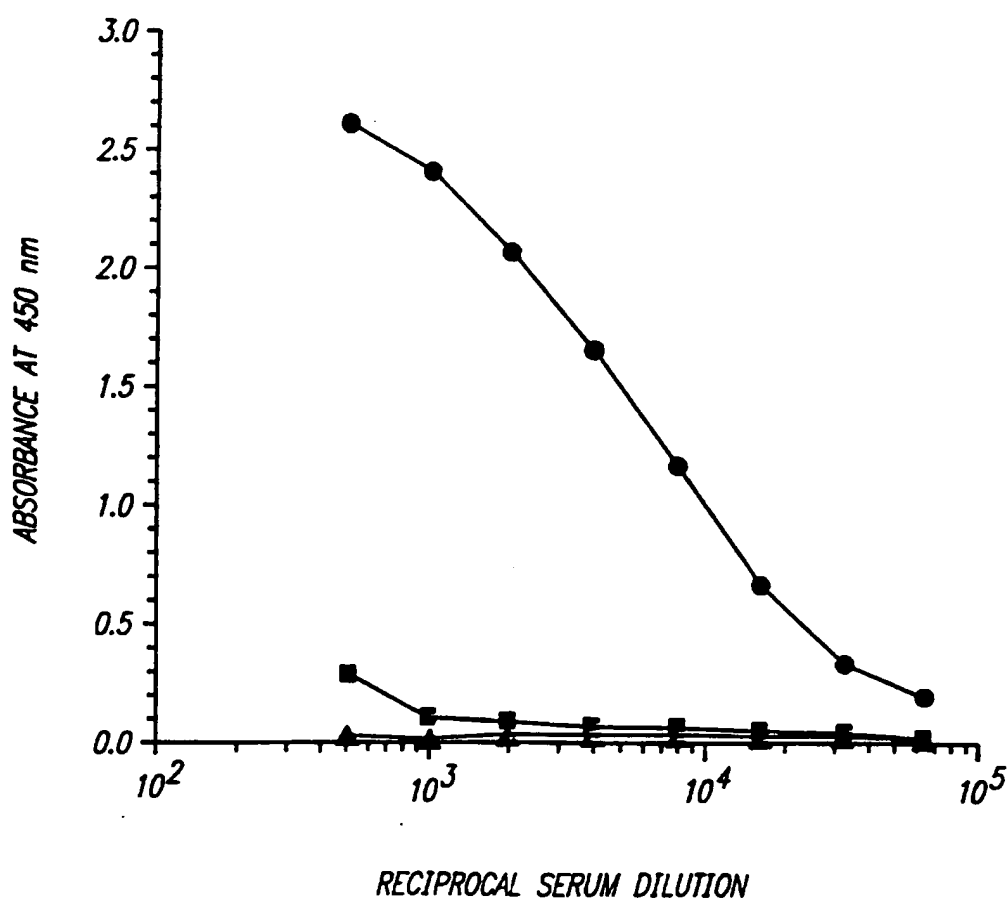


FIG. 13A

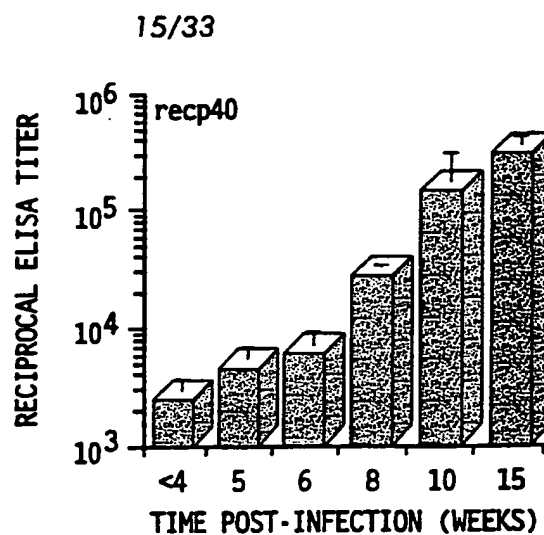


FIG. 13B

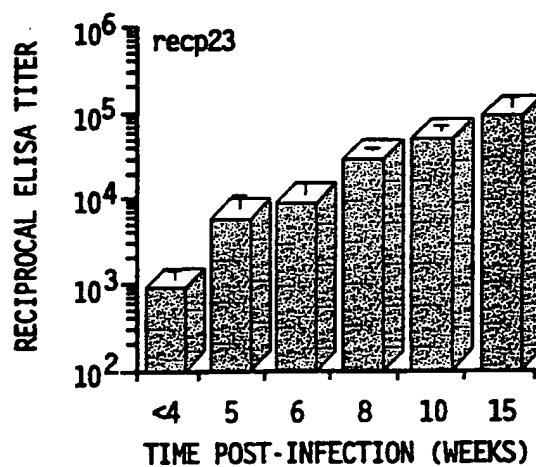
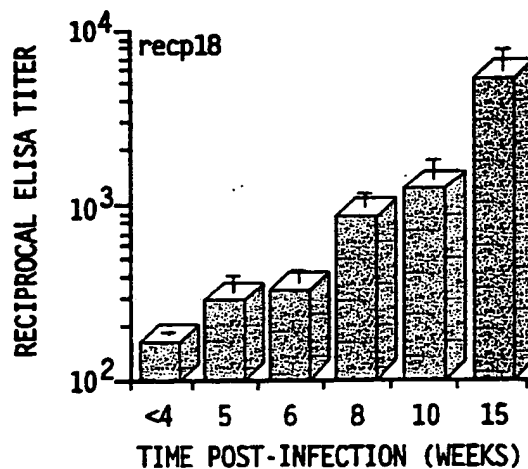


FIG. 13C





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FIG. 14A

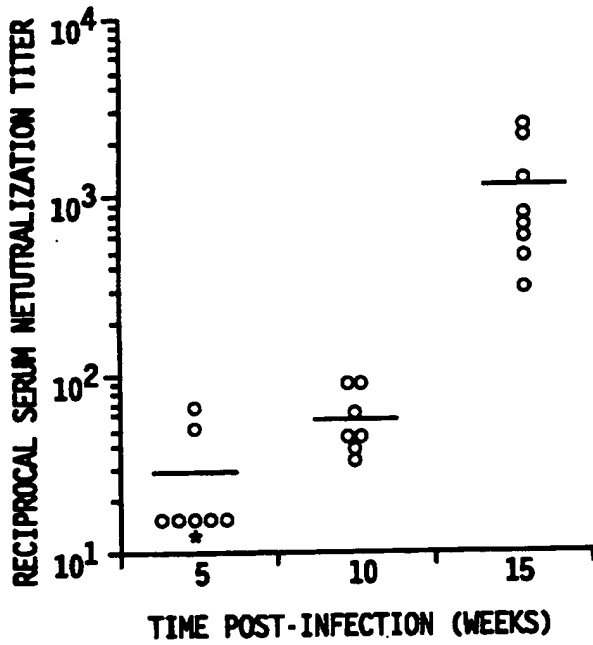


FIG. 14C

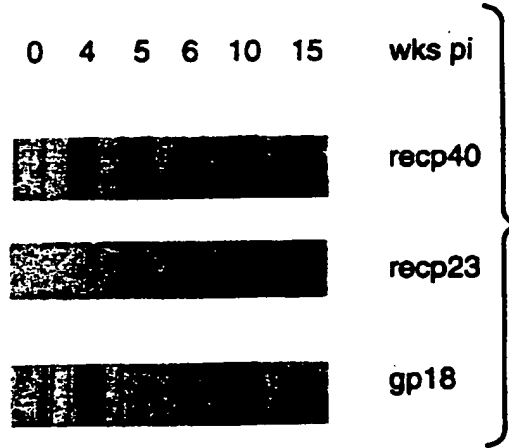


FIG. 14B

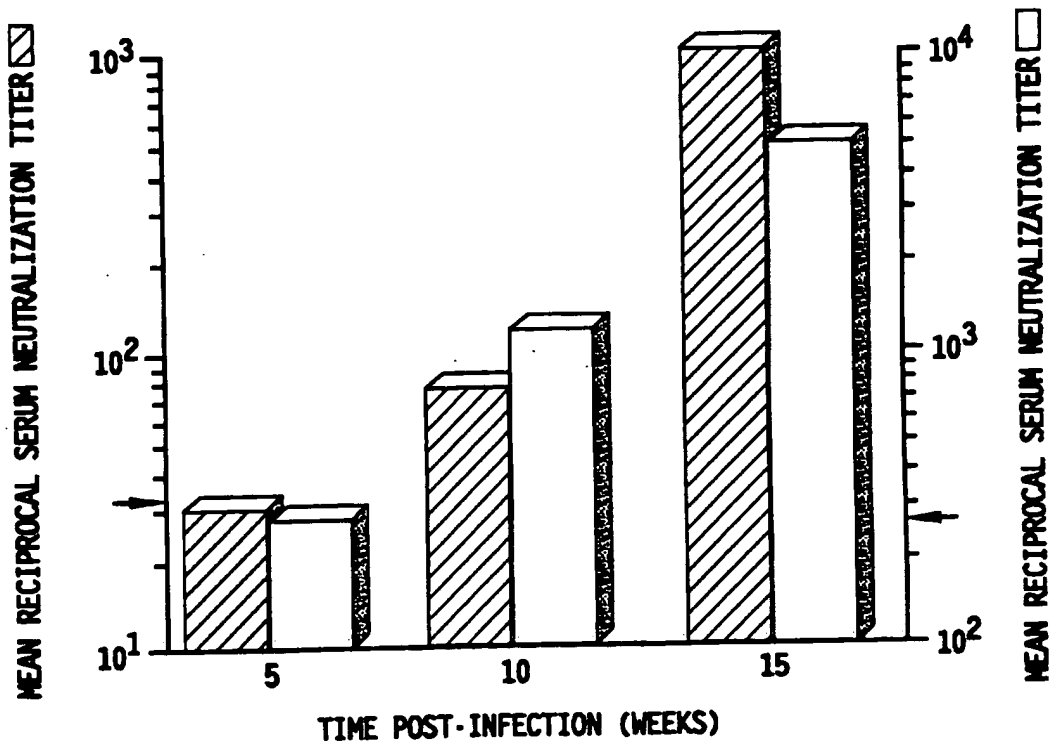


FIG. 15A



FIG. 15B

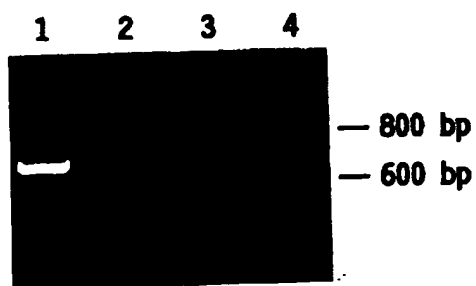
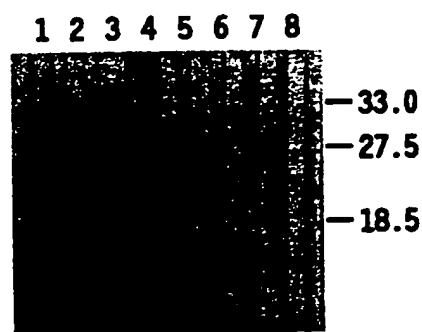


FIG. 17A

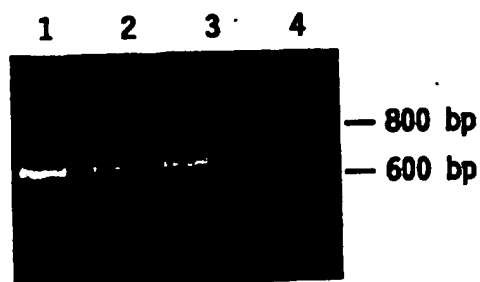


FIG. 17B

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FIG. 16A

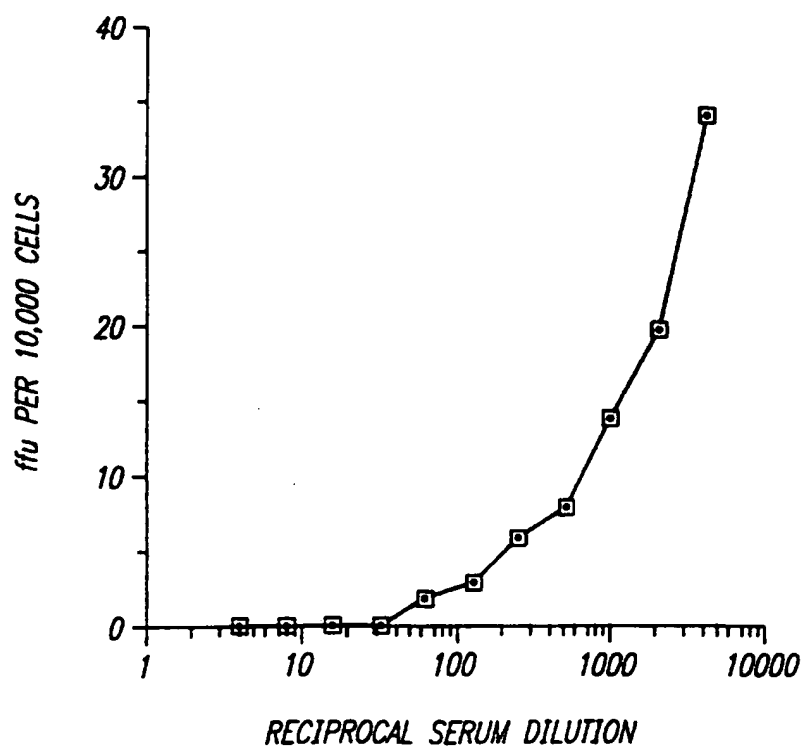
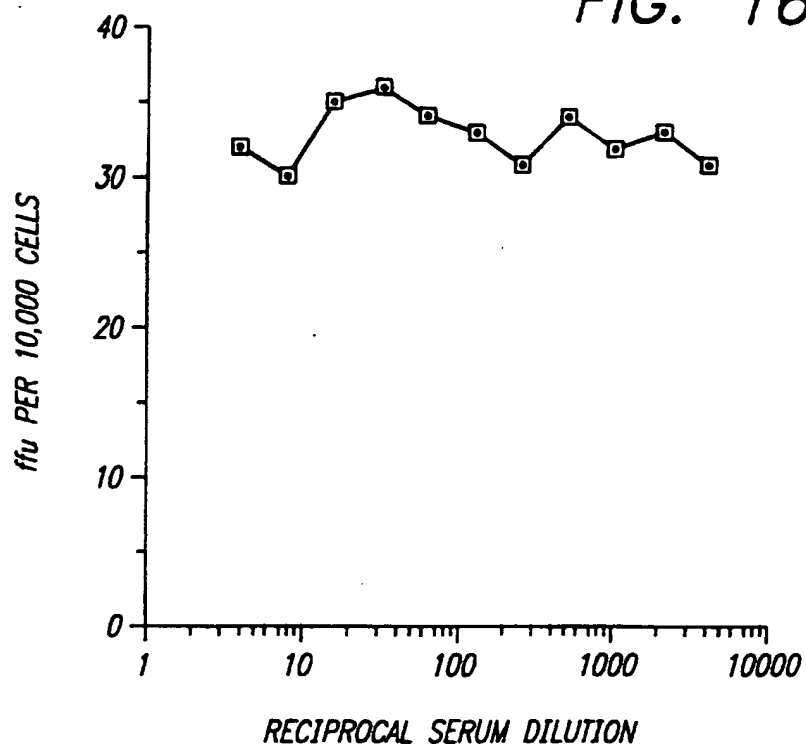


FIG. 16B

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FIG. 16C

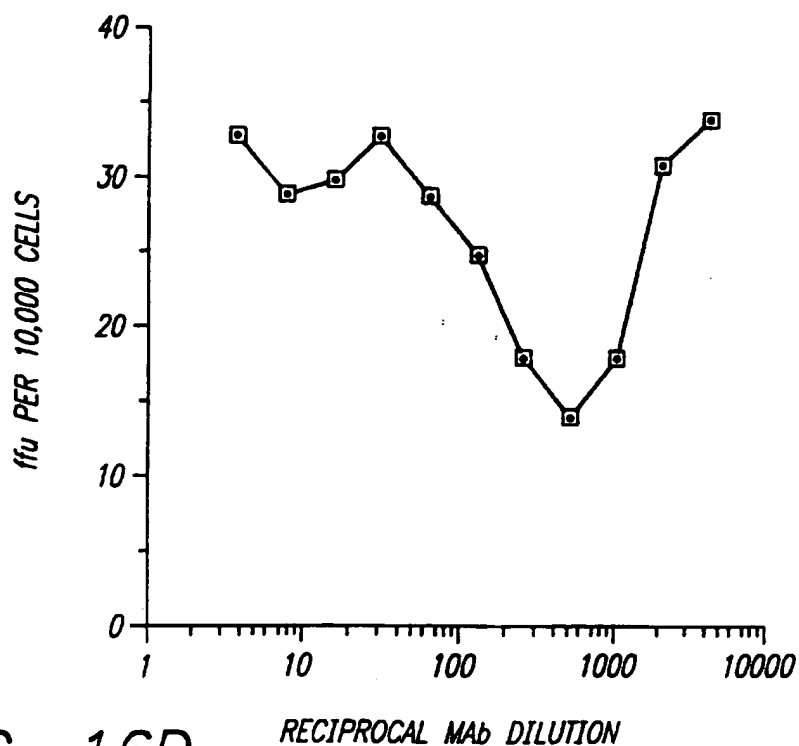
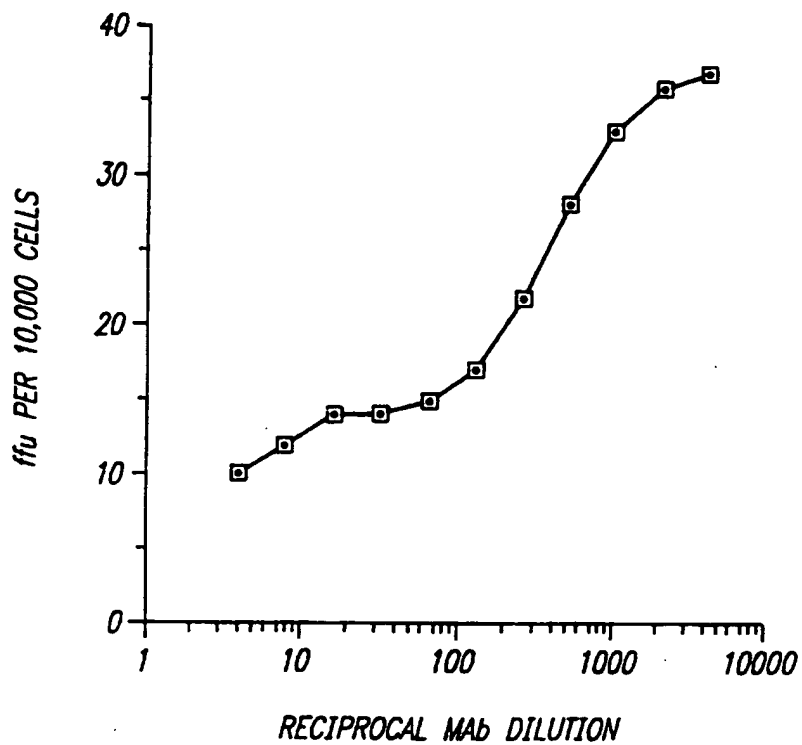


FIG. 16D

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## POLYMERASE NUCLEOTIDE SEQUENCE

FIG. 18(1)

1-2  
2393 ATGTCATTTTCATGCGAG\ / CCTCCTTCGCGAGGAGGAGACGCCTCGGCCGGTGGCAGGA 3743  
-----+-----+ V -----+-----+-----+-----+-----  
TACAGTAAAGTACGCTC GGAGGAAGCGCTCCTCCTCTGCGGAGCCGGCCACCGTCCT  
3744 ATAAACCGTACCGACCACTCTCTTAAAAACCCCTCTCCTCGGAACAGAGGTCTCTTTCTGC 3803  
-----+-----+-----+-----+-----+-----+-----  
TATTTGGCATGGCTGGTCAGAGAATTTTGGGAGAGGAGCCTTGTCTCCAGAGAAAGACG  
3804 CTTAAGTCGAGCTCACTCCCCATCATGTACGAGCACTAGGCCAGATTAAAGCAAGGAAC 3863  
-----+-----+-----+-----+-----+-----+-----  
GAATTCAGCTCGAGTGAGGGGGTAGTACATGCTCGTGATCCGGTCTAATTCGTTCTTG  
3864 CTGGCATCCTGTGACTATTACTTGCTATTCCGCCAAGTTGTATTGCCCCCTGAAGTATAT 3923  
-----+-----+-----+-----+-----+-----+-----  
GACCGTAGGACACTGATAATGAACGATAAGGCGGTTCAACATAACGGGGGACTTCATATA  
3924 CCCATTGGTGTCTAATAAGAGCTGCGGAGGCTATACTAACAGTTATAGTATCAGCTTGG 3983  
-----+-----+-----+-----+-----+-----+-----  
GGGTAACCACAAGATTATTCTCGACGCCTCCGATATGATTGTCAATATCATAGTCGAACC  
3984 AAGCTGGATCATATGACGAAGACCCTATACTCCTCTGTGAGATATGCACTCACCAATCCC 4043  
-----+-----+-----+-----+-----+-----+-----  
TTGACCTAGTATACTGCTTCTGGGATATGAGGAGACACTCTATACGTGAGTGGTTAGGG  
4044 CGGGTCCGAGCCCAACTTGAGCTTCACATTGCCTACCAGCGCATAGTGGGTCAAGTCTCG 4103  
-----+-----+-----+-----+-----+-----+-----  
GCCCAGGCTCGGGTTGAACTCGAAGTGTAACGGATGGTCGCGTATCACCAGTCCAGAGC  
4104 TACAGCCGGGAGGCAGACATAAGGGCCAAAAAGGCTTGGGAATATGTCATTGCAATTCATC 4163  
-----+-----+-----+-----+-----+-----+-----  
ATGTCGGCCCTCCGTCTGTATCCCGGTTTTTCCGAACCTTATACAGTAACGTAAAGTAG  
4164 CAATCTCTCGTTATTGCCACCATAGACACGACAAGCTGCCTAATGACCTACAACCACTTT 4223  
-----+-----+-----+-----+-----+-----+-----  
GTTAGAGAGCAATAACGGTGGTATCTGTGCTGTTGACGGATTACTGGATGTTGGTGAAG  
4224 CTTGCTGCAGCAGACACAGCCAAGAGCAGATGCCATCTCCTAATCGCCTCAGTGGTCCAG 4283  
-----+-----+-----+-----+-----+-----+-----  
GAACGACGTCGTCTGTGTCGGTTCTCGTCTACGGTAGAGGATTAGCGGAGTCAACAGGTC  
4284 GGGGCCCTTTGGGAACAAGGGTCATTTCTTGATCATATAATCAACATGATCGACATAATT 4343  
-----+-----+-----+-----+-----+-----+-----  
CCCCGGGAAACCCTTGTCCAGTAAAGAACTAGTATATTAGTTGTACTAGCTGTATTAA  
4344 GACTCAATCAACCTCCCCATGATGATTACTTCACAATTATTAAGTCTATCTTTCCCTAC 4403  
-----+-----+-----+-----+-----+-----+-----  
CTGAGTTAGTTGGAGGGGGTACTACTAATGAAGTGTTAATAATTCAGATAGAAAGGGATG  
4404 TCCCAAGGGCTTGTATGGGGAGGCATAATGTATCAGTCTCCTCTGATTTGCGGTCCGTA 4463  
-----+-----+-----+-----+-----+-----+-----  
AGGGTTCCCGAACAATACCCCTCCGTATTACATAGTCAGAGGAGACTAAAGCGCAGGCAT  
4464 TTTGCCATTCTGAATTATGCCCGCAACTAGACAGCTTACTAAAAAACTGCTCCAACCTT 4523  
-----+-----+-----+-----+-----+-----+-----  
AAACGGTAAGGACTTAATACGGGCGTTGATCTGTGCAATGATTTTTTTGACGAGGTTGAA  
4524 GACCCCGTTCTCCTCCTCATGGTCTCTTCCGGTGCAGAAGTCATGGTACTTCCCTGAGATC 4583  
-----+-----+-----+-----+-----+-----+-----  
CTGGGGCAAGAGGAGGAGTACCAGAGAAGCCACGTCTTCAGTACCATGAAGGGACTCTAG



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## FIG. 18(3)

5544 AAAACAATGGGGAGGGCATGAGGCAGAAACTATGGACAATCCTTACGAGCTGCTGGGAG 5603  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
TTTTGTTACCCCTCCCGTACTCCGTCTTTGATACCTGTTAGGAATGCTCGACGACCCTC  
5604 ATAATTGCTCTTCGGGAAATTAACGTGACGTTTAACTACTAGGCCAAGGTGATAATCAG 5663  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
TATTAACGAGAAGCCCTTTAATTGCACTGCAAATTGTATGATCCGGTCCACTATTAGTC  
5664 ACAATCATCATACATAAATCTGCAAGCCAAAATAACCAGCTATTAGCGGAGCGAGCACTA 5723  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
TGTTAGTAGTATGTATTTAGACGTTCCGGTTTTATTGGTCGATAATCGCCTCGCTCGTGAT  
5724 GGGGCCCTGTACAAGCATGCTAGATTAGCTGGCCATAACCTCAAGGTAGAGGAATGCTGG 5783  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
CCCCGGGACATGTTCTGACGATCTAATCGACCGGATTGGAGTTCATCTCCTTACGACC  
5784 GTGTCAGATTGTCTGTATGAGTATGGAAAGAAGCTTTTCTTCGGTGGTGTACCTGTCCCG 5843  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
CACAGTCTAACAGACATACTCATACCTTTCTTCGAAAAGAAGGCACCACATGGACAGGGC  
5844 GGCTGTTTGAAGCAGCTCTCACGGGTGACGGATTCTACTGGAGAGCTATTCCCAAACCTA 5903  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
CCGACAAACTTCGTCGAGAGTGCCCACTGCCTAAGATGACCTCTCGATAAGGGTTTGGAT  
5904 TACTCAAAGTTAGCCTGCTTAACATCATCGTGTTTAAGCGCAGCGATGGCAGACACATCT 5963  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
ATGAGTTTCAATCGGACGAATTGTAGTAGCACAATTGCGTCGCTACCCTGTGTAGTA  
5964 CCATGGGTGGCACTCGCAGAGGTGTCTGTCTGTATCTTATCGAGTTATATGTTGAGCTG 6023  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
GGTACCCACCGTGAGCGCTGTCCACAGACAGACATAGAATAGCTCAATATACAACCTCGAC  
6024 CCTCCAGCAATCATGCAGGATGAGTCGCTATTGACGACCCTCTGCCTCGTAGGGCCATCC 6083  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
GGAGGTCGTTAGTACGTCTACTCAGCGATAACTGCTGGGAGACGGAGCATCCGGGTAGG  
6084 ATTGGTGGGCTTCGACCCCTGCAACCCTACCCAGTGCTTTTTTCAGAGGAATGTCCGAC 6143  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
TAACCACCCGAAAGGCTGGGGACGTTGGGATGGGTACAGAAAAAGTCTCCTTACAGGCTG  
6144 CCACTGCCCTTTCACTAGCACTCTTGCAAGCCCTCATTAAAGACGACAGGGGTGACCTGT 6203  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
GGTGACGGGAAAGTCGATCGTGAGAACGTCTGGGAGTAATTCTGCTGTCCCCACTGGACA  
6204 AGCTTGGTGAATCGTGTGGTCAAGTTACGGATAGCACCTATCCAGACTGGCTCTCTCTA 6263  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
TCGAACCACTTAGCACACCAGTTCAATGCCTATCGTGGGATAGGTCTGACCGAGAGAGAT  
6264 GTGACTGACCCGACCTCACTCAACATTGCCCAAGTGATCCGGCCAGAACGTCAGATCAGG 6323  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
CACTGACTGGGCTGGAGTGAGTTGTAACGGGTTACATGGCCGGTCTTGCACTCTAGTCC  
6324 AGGTGGATTGAGGAAGCGATAGCGACAAGCTCACACTCGTCACGCATAGCAACTTTCTTC 6383  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
TCCACCTAACTCCTTCGCTATCGCTGTTGAGTGAGCAGTGCATCGTTGAAAGAAG  
6384 CAGCAGCCCTCACGGAGATGGCTCAGTTGCTTGCGAGGGACCTTTCAACAATGATGCCT 6443  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
GTCGTGCGGGAGTGCTCTACCGAGTCAACGAACGCTCCCTGGAAAGTTGTTACTACGGA  
6444 CTTGACCCCGGGATATGTCGGCCTTATTCGATTATCAAATGTCGCATACGGTTTAAAGC 6503  
-----+-----+-----+-----+-----+-----+-----+-----+-----  
GAAGCTGGGGCCCTATACAGCCGGAATAAGCGTAATAGTTTACAGCGTATGCCAAATTGC

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## FIG. 18(4)

6504 ATTATAGATCTATTTCAAAAATCCTCTACCGTTGTTTCTGCAAGTCAAGCTGTCCATATC 7563  
-----+-----+-----+-----+-----+-----+-----+-----  
TAATATCTAGATAAAGTTTTAGGAGATGGCAACAAAGACGTTTCAGTTCGACAGGTATAG

6564 GAGGATGTTGCCCTAGAGAGTGTAAAGGTATAAGGAATCTATCATCCAGGGTCTGTAGAC 7623  
-----+-----+-----+-----+-----+-----+-----+-----  
CTCCTACAACGGGATCTCTCACATTCCATATTCCTTAGATAGTAGGTCCCAGACAATCTG

6624 ACCACTGAGGGGTATAACATGCAACCTTATTTGGAAGGTTGCACTTACCTTGACGCCAAA 7683  
-----+-----+-----+-----+-----+-----+-----+-----  
TGGTGACTCCCCATATTGTACGTTGGAATAAACCTTCCAACGTGAATGGAACGTCGGTTT

6684 CAGTTACGTAGGTTGACATGGGGTCGAGACCTAGTTGGAGTCACAATGCCGTTTGTGACC 7743  
-----+-----+-----+-----+-----+-----+-----+-----  
GTCAATGCATCCAACCTGTACCCAGCTCTGGATCAACCTCAGTGTTACGGCAAACAACGG

6744 GAGCAATTCATCCTCACAGTTCTGTGGGTGCAAAGGCGGAACCTACCTCGACGCTATT 7803  
-----+-----+-----+-----+-----+-----+-----+-----  
CTCGTTAAGGTAGGAGTGTCAAGACACCCACGTTTCCGCCCTTGAGATGGAGCTGCGATAA

6804 ATATACTGCCACAGGAGACATTGCGGTACACCATCTGACTACCAGGGGGGACCAGCCG 7863  
-----+-----+-----+-----+-----+-----+-----+-----  
TATATGACGGGTGCTCTGTAAACGCCAGTGTGGTAGACTGATGGTCCCCCTGGTGGC

6864 CTTTACCTCGGATCCAATACGGCTGTCAAGGTCCAGCGAGGTGAGATCACGGGCTAACA 7923  
-----+-----+-----+-----+-----+-----+-----+-----  
GAAATGGAGCCTAGGTTATGCCGACAGTTCAGGTGCTCCACTCTAGTGCCCGGATTGT

6924 AAGTCAAGGGCTGCAAACTAGTCAGGGACACTCTCGTTCTCCATCAGTGGTATAAAGTC 7983  
-----+-----+-----+-----+-----+-----+-----+-----  
TTCAGTTCCCGACGTTTAGATCAGTCCCTGTGAGAGCAAGAGGTAGTCACCATATTTAG

6984 CGTAAAGTTACCGATCCACACTTGAACACCCTCATGGCAGCTTCTTACTTGAGAAGGGG 7043  
-----+-----+-----+-----+-----+-----+-----+-----  
GCATTTCAATGGCTAGGTGTGAACCTGTGGGAGTACCGTGGGAAGATGAACCTTCCCC

7044 TACACATCTGACGCTCGACCTAGCATCCAGGGTGGGACCTCACGCATCGTCTCCCATCC 7103  
-----+-----+-----+-----+-----+-----+-----+-----  
ATGTGTAGACTGGGAGCTGGATCGTAGGTCCCACCCTGGGAGTGCCTAGCAGAGGGTAGG

7104 CGCGGAGACTCACGGCAGGGGCTTACTGGGTATGTAATATACTAAGTACGTGGCTTCGA 7163  
-----+-----+-----+-----+-----+-----+-----+-----  
GCGCCTCTGAGTGCCGTCCCGAATGACCCATACATTTATATGATTCATGCACCGAAGCT

7164 TTCTCAAGTGATTATCTTCACTCTTTCTCGAAATCATCAGACGACTATACAATCCACTTT 7223  
-----+-----+-----+-----+-----+-----+-----+-----  
AAGAGTTCACTAATAGAAGTGAGAAAGAGCTTTAGTAGTCTGCTGATATGTTAGGTGAAA

7224 CAGCATGTATTACATACGGTTGCCTCTATGCTGATTGGTGATTAGATCGGGCGGTGTT 7283  
-----+-----+-----+-----+-----+-----+-----+-----  
GTCGTACATAAGTGATGCCAACGGAGATACGACTAAGCCACTAATCTAGCCCCGCCACAA

7284 ATTTCACTCCTTACCTTTTGAAGTGAAGTTGTAAAACATGCTTTGAGAAGATAGACTCA 7343  
-----+-----+-----+-----+-----+-----+-----+-----  
TAAAGGTGAGGAATGGAAAACCTCACGTTCAACATTTTGTACGAACTCTTCTATCTGAGT

7344 GAGGAGTTCGTCTGCGATGTGAACCCCAATACAGGGGTGCTGAGTGGCTGATATCAAAG 7403  
-----+-----+-----+-----+-----+-----+-----+-----  
CTCCTCAAGCAGGACCGTACACTTGGGGTTATGTCGCCACGACTCACCAGCTATAGTTTC

7404 CCAGTCACTGTCCCTGAGCAGATAACTGATGCTGAAAGTCGAGTTTGACCCCTGTGTGAGT 7463  
-----+-----+-----+-----+-----+-----+-----+-----  
GGTCAGTGACAGGGACTCGTCTATTGACTACGACTTCAGCTCAAACCTGGGGACACACTCA



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## FIG. 18(5)

7464 GC GGGTTATTGTCTCGGGATTCTCATTGGCAAGTCATTCTTAGTTGACATAAGGGCAAGT 7523  
CGCCCAATAACAGAGCCCTAAGAGTAACCGTTCAGTAAGAATCAACTGTATTCCCGTTCA  
7524 GGGCATGATATCATGGAGCAGCGGACATGGGCTAACCTGGAGAGGTTTTCTGTATCGGAC 7583  
CCCCTACTATAGTACCTCGTCGCCTGTACCCGATTGGACCTCTCCAAAAGACATAGCCTG  
7584 ATGCAGAACTTCCGTGGAGTATTGTAATTCGGTCTCTCTGGAGATTCTTATTGGCGCA 7643  
TACGTCTTTGAAGGCACCTCATAACATTAAGCCAGAGAGACCTCTAAGGAATAACCGCGT  
7644 CGGCTCCTTCAGTTTGAGAAGGCTGGCCTCATTAGAATGCTGTATGCTGCGACAGGTCCA 7703  
GCCGAGGAAGTCAAACCTTCCGACCGGAGTAATCTACGACATACGACGCTGTCCAGGT  
7704 ACCCCTAGCTTCTAATGAAAGTTTTTCAAGACTCAGCCCTCTCATGGACTGCGACCCC 7763  
TGGGGATCGAAGGATTACTTTCAAAAGTTCTGAGTCGGGAGGAGTACCTGACGCGTGGG  
7764 CTCGATCGGCTGTCCCCTAGGATCAACTTTCATAGTCGGGGAGACCTCGTTGCTAAGCTT 7823  
GAGCTAGCCGACAGGGGATCCTAGTTGAAAGTATCAGCCCTCTGGAGCAACGATTTCGAA  
7824 GTTTTATTGCCCTTCATCAACCCGGGTATAGTGGAGATTGAAGTGTCTGGAATTAATAGC 7883  
CAAAATAACGGGAAGTAGTTGGGCCCATATCACCTCTAACTTCACAGACCTTAATTATCG  
7884 AAGTACCATGCAGTATCGGAGGCCAATATGGATCTGTACATCGCTGCTGCCAAGTCTGTG 7943  
TTCATGGTACGTATAGCCTCCGGTTATACCTAGACATGTAGCGACGACGGTTCAGACAC  
7944 GCGGTGAAGCCACACAGTTTGTGAGGAAACAAACGACTTTACGGCCCGCGGCCACCAC 8003  
CCGCACTTCGGGTGTGTCAAACAACCTCCTTTGTTTGTGAAATGCCGGCGCCGGTGTTG  
8004 CATGGTTGTTATTCCTTTCTTGGTCTAAGTCACGCAATCAATCACAGGTCCTAAAGATG 8063  
GTACCAACAATAAGGGAAAGAACCAGATTCAAGTGCCTAGTTAGTGTCCAGGATTTCTAC  
8064 GTAGTACGGAAGCTGAAGCTCTGTGCTCTGTATATATACCCACAGTCGATCCCGCGGTT 8123  
CATCATGCCCTTCGACTTCGAGACACAGGACATATATATGGGGTGTCAAGTACGGGCGGCAA  
8124 GCTCTCGACCTGTGCCATCTACCAGCATTAACTATAATCCTAGTGTCTGGCGGTGACCCA 8183  
CGAGAGCTGGACACGGTAGATGGTCGTAATTGATATTAGGATCACGAGCCGCCACTGGGT  
8184 GCGTACTATGAGCGATTACTTGAGATGGACCTGTGCGGGGCTGTGTCAAGTCGAGTCGAT 8243  
CGCATGATACTCGCTAATGAACCTCTACCTGGACACGCCCCGACACAGTTCAGCTCAGCTA  
8244 ATCCCCATTCTCTGGCTGGCAGAACGCACAGGGGGTTCGAGTGGGCCCAGACGCTGGT 8303  
TAGGGGGTAAGAGACCGACCGTCTTGCCTGTCCCCAAGCGTCACCCGGGTCTGCGACCA  
8304 CCAGGTGTAATTAGACTCGACAGGTTAGAGTCAGTTTGTATGCTCACCCCTGTTTAGAG 8363  
GGTCCACATTAATCTGAGCTGTCCAATCTCAGTCAAACAATACGAGTGGGGACAAATCTC  
8364 GAACTAGAGTTTAATGCATATCTAGACTCTGAGTTGGTTGACATTAGTGATATGTGCTGC 8423  
CTTGATCTCAAATTACGTATAGATCTGAGACTCAACCAACTGTAATCACTATACACGACG

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FIG. 18(6)

8424 CTCCCCTTAGCGACACCCTGTAAGGCCCTTTTCAGGCCAATATATCGGAGCTTACAGTCG 8483  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GAGGGGAATCGCTGTGGGACATTCCGGGAAAAGTCCGGTTATATAGCCTCGAATGTCAGC  
8484 TTCAGGTTAGCCTTAATGGACAACTATAGTTTTGTCATGGACCTCATTATGATCCGAGGA 8543  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
AAGTCCAATCGGAATTACCTGTTGATATCAAAACAGTACCTGGAGTAATACTAGGCTCCT  
8544 CTGGACATTAGGCCTCACCTTGAGGAATTTGACGAGCTGCTTGTGGTAGGACAGCACATC 8603  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GACCTGTAATCCGGAGTGGAACCTCTTAACTGCTCGACGAACACCATCCTGTCGTGTAG  
8604 CTCGGCCAGCCCCTCCTAGTAGAGGTTGTTTACTACGTTGGAGTTGTTAGGAAGCGCCCT 8663  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GAGCCGGTCGGGCAGGATCATCTCCAACAAATGATGCAACCTCAACAATCCTTCGCGGGA  
8664 GTGTTAGCGAGGCATCCGTGGTCAGCAGATCTTAAGCGAATTACTGTGGGGGGGCGGCCT 8723  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
CACAATCGCTCCGTAGGCACCACTGCTCTAGAATTCGCTTAATGACACCCCCCGCCGA  
8724 CCCTGCCCCTCTGCTGCCAGATTGCGTGATGAGGATTGTCAGGGGCTCTGTTGGTTGGG 8783  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GGGACGGGAGACGACGGTCTAACGCACTACTCCTAACAGTCCCAGAGACAACCAACC  
8784 CTTCTGCTGGGTTGACGCACTTATTGATAATTGATTA 8821  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GAAGGACGACCAACTGCGTCAATAACTATTAATAAT

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## FIG. 19(1)

1 GGTAGACCAG CTCCTGAAGA ACCTCAGGAA GAACCCCTCC ATGATCTCAG  
51 ACCCAGACCA GCGAACCGGA AGGGAGCAGC TATCGAATGA TGAGCTTATC  
101 AAGAAGCTAG TGACGGAGCT GGCCGAGAAT AGCATGATCG AGGCTGAGGA  
151 GGTGCGGGGC ACTCTTGGGG ACATCTCGGC TCGCATCGAG GCAGGGTTTG  
201 AGTCCCTGTC CGCCCTCCAA GTGGAAACCA TCCAGACAGC TCAGCGGTGC  
251 GACCACTCCG ATAGCATCAG AATCCTTGGC GAGAACATCA AGATACTGGA  
301 TCGCTCCATG AAGACAATGA TGGAGACAAT GAAGCTCATG ATGGAGAAGG  
351 TGGACCTCCT CTACGCATCA ACCGCCGTTG GGACCTCTGC ACCCATGTTG  
401 CCCTCCCATC CTGCACCTCC GCGCATTTAT CCCAGCTCC CAAGTGCCCC  
451 GACAGCGGAT GAGTGGGACA TCATACCATA AAAAAATCGA ATCACCATGA  
501 ATTCAAAGCA TTCCTATGTG GAGCTCAAGG ACAAGGTAAT CGTCCCTGGA  
551 TGGCCCACAC TGATGCTTGA GATAGACTTT GTAGGAGGGA CTTACGGAA  
601 CCAGTTCCTT AACATCCCAT TTCTTTCAGT GAAAGAGCCT CTGCAGCTTC  
651 CACGCGAGAA GAAGTTGACC GACTACTTCA CCATTGACGT AGAGCCAGCA  
701 GGTCAATCCC TGGTCAACAT ATACTTCCAG ATTGACGACT TCTTGCTCCT  
751 AACACTCAAC TCACTGTCCG TATACAAGGA CCCGATTAGG AAATACATGT  
801 TCCTACGCCT CAACAAGGAA CAGAGCAAGC ACGCAATTAA TGCAGCTTTC  
851 AATGTCTTCT CTTATCGGCT TCGGAACATT GGTGTTGGCC CTCTCGGCCC  
901 AGACATTCGA TCTTCAGGGC CTTAGTTGCA ATACTGACTC CACTCCTGGA  
951 TTAATCGATC TGGAGATAAG GCGACTTTGA CACACCCCAA CGGAAAATGT  
1001 CATTTTCATGC GAGGTTAGTT ATCTTAACCA CACGACTATT AGCCTCCCGG  
1051 CAGTCCACAC GTCATGCCTC AAGTACCACT GCAAAACCTA TTGGGGATTG  
1101 TTTGGTAGCT ACAGCGCTGA CCGAATCATC AATCGGTACA CTGGTACTGT  
1151 TAAGGGTTGT TTAAACAAC T CAGCGCCAGA GGATCCCTTC GAGTGCAACT  
1201 GGTTCCTACTG CTGCTCGGCG ATTACAACAG AGATCTGCCG ATGCTCTATT  
1251 ACAAATGTCA CGGTGGCTGT ACAGACATTC CCACCGTTCA TGTACTGCAG  
1301 TTTCGCGGAC TGTAGTACTG TGAGTCAGCA GGAGCTAGAG AGTGGAAGG  
1351 CAATGCTGAG CGATGGCAGT ACCTTAACTT ATACCCCGTA TATCTTACAA  
1401 TCAGAAGTCG TGAACAAAAC CCTTAATGGG ACTATACTCT GCAACTCATC  
1451 CTCCAAGATA GTTTCCTTCG ATGAATTTAG GCGTTCATAC TCCCTAGCGA

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## FIG. 19(2)

1501 ATGGTAGTTA CCAGAGCTCA TCAATCAATG TGACGTGTGT AA ACTACACG  
1551 TCGTCCTGCC GGTCCAAGTT GAGAAGGCGG CGTAGGGATA CTCAACAGAT  
1601 TGAGTACCTA GTTCACAAGC TTAGGCCTAC ACTGAAAGAT GCGTGGGAGG  
1651 ACTGTGAGAT CCTCCAGTCT CTGCTCCTAG GGGTGTTTGG TACTGGGATT  
1701 GCAAGTGCTT CGCAATTCTT GAGGGGCTGG CTCAACCACC CTGATATCAT  
1751 CGGGTATATA GTTAATGGAG TTGGGGTAGT CTGGCAATGC CATCGTGTG  
1801 ATGTCACGTT CATGGCGTGG AATGAGTCCA CATATTACCC TCCAGTAGAT  
1851 TACAATGGAC GGAAGTACTT TCTGAATGAT GAGGGGAGGC TACAAACAAA  
1901 CACCCCCGAG GCAAGGCCAG GGCTTAAGCG GGT CATGTGG TTCGGCAGGT  
1951 ACTTCCTAGG GACAGTAGGG TCTGGGGTGA AACCGAGGAG GATT CGGTAC  
2001 AATAAGACCT CACATGATTA CCATCTAGAG GAGTTTGAGG CAAGTCTCAA  
2051 CATGACCCCC CAGACCAGTA TCGCCTCGGG TCATGAGACA GACCCCATAA  
2101 ATCATGCCTA CGGAACGCAG GCTGACCTCC TTCCATACAC CAGGTCTAGT  
2151 AATATAACGT CTACAGATAC AGGCTCAGGC TGGGTGCACA TCGGCCTACC  
2201 CTCATTTGCT TTCCTCAATC CTCTCGGGTG GCTTAGGGAC CTACTTGCGT  
2251 GGGCGGCCTG GTTGGGTGGG GTTCTATACT TAATAAGTCT TTGTGTTTCC  
2301 TTACCAGCCT CCTTCGCGAG GAGGAGACGC CTCGGCCGGT GGCAGGAATA  
2351 AACCGTACCG ACCAATCTCT TAAAAACCCT CTTCTCGGGA CAGAGGTCTC  
2401 TTTCTGCCTT AAATCGAGTT CACTCCCCCA TCACGTACGA GCATTGGGCC  
2451 AGATTAAAGC AAAGAACCTG GCATCCTGTG ACTATTACTT GCTATTCCGC  
2501 CAAGTTGTAT TGCCCCCTGA AGTATATCCC ATTGGTGTCT TAATAAGAGC  
2551 TGCGGAGGCC ATACTAACAG TTATAGTATC AGCTTGGAAG CTGGATCACA  
2601 TGACAAAGAC CCTATACTCC TCTGTGAGAT ATGCACTCAC CAATCCCCGG  
2651 GTCCGGGC

FIG. 20A

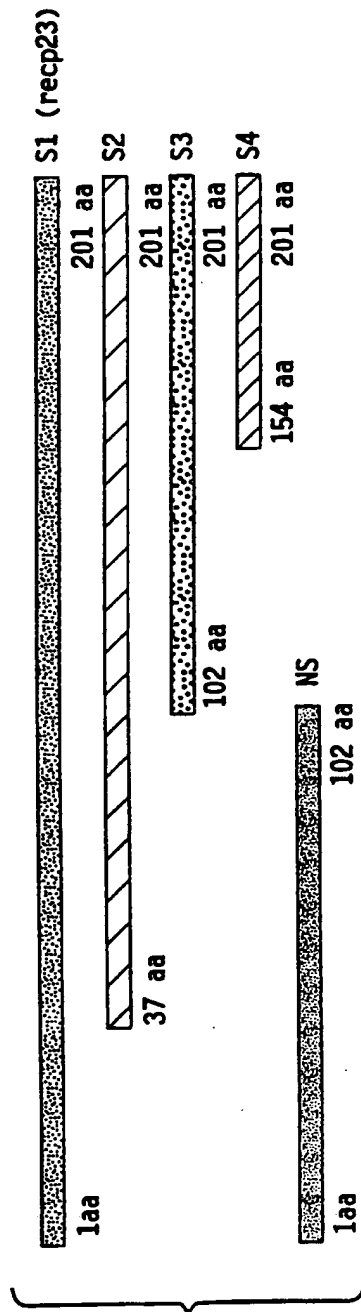
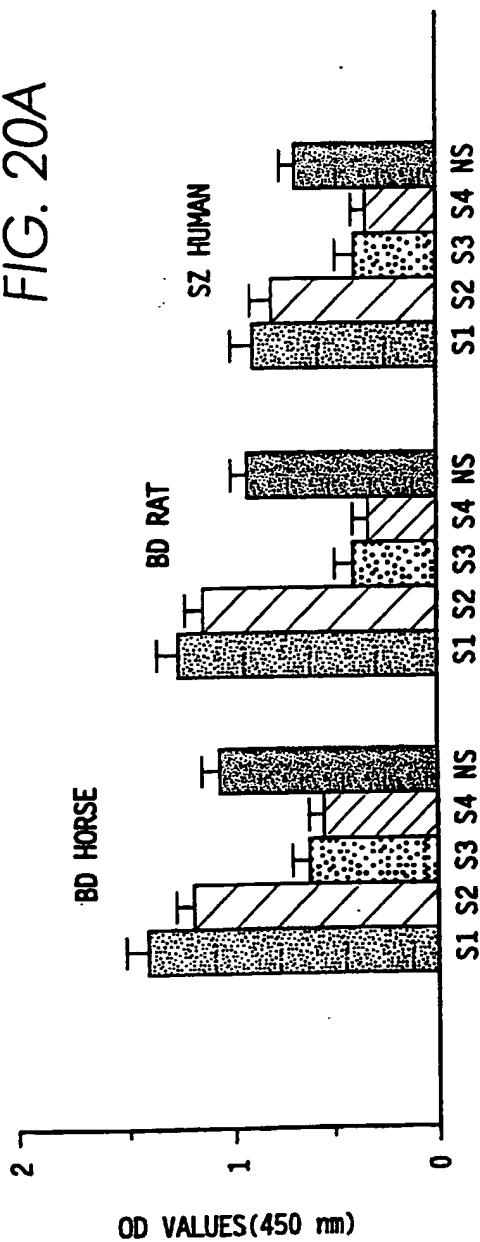


FIG. 20B

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FIG. 21A

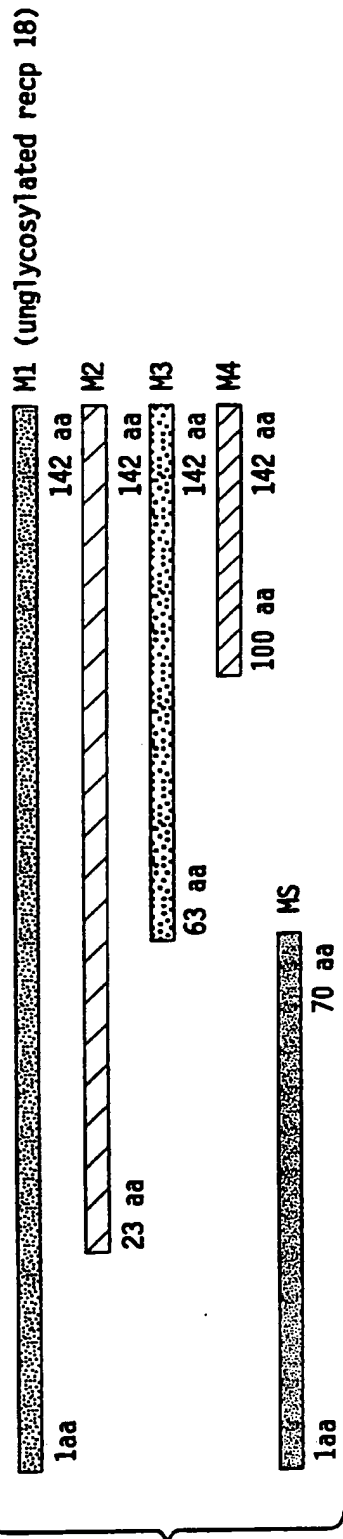
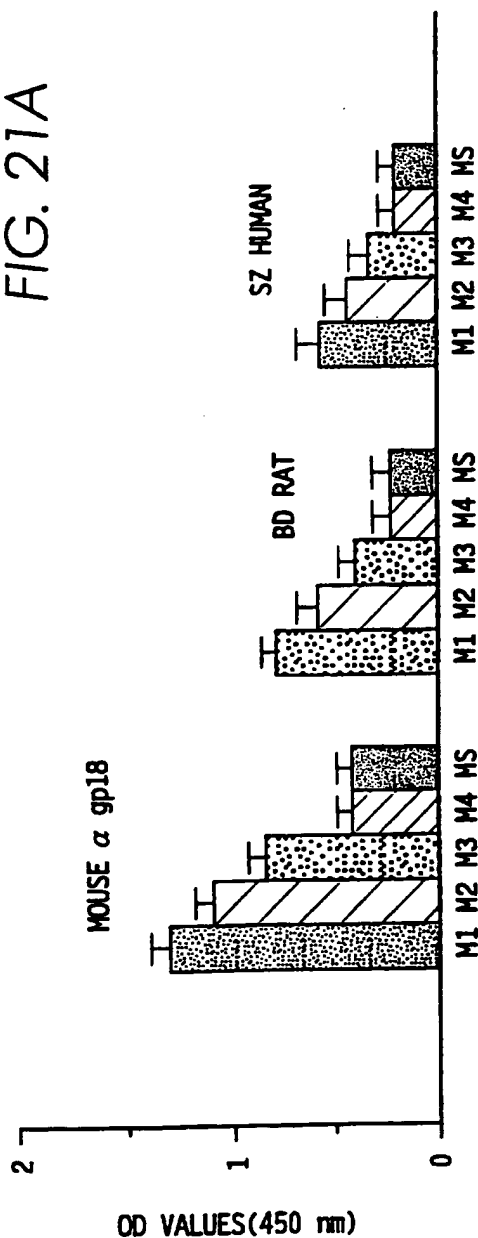


FIG. 21B

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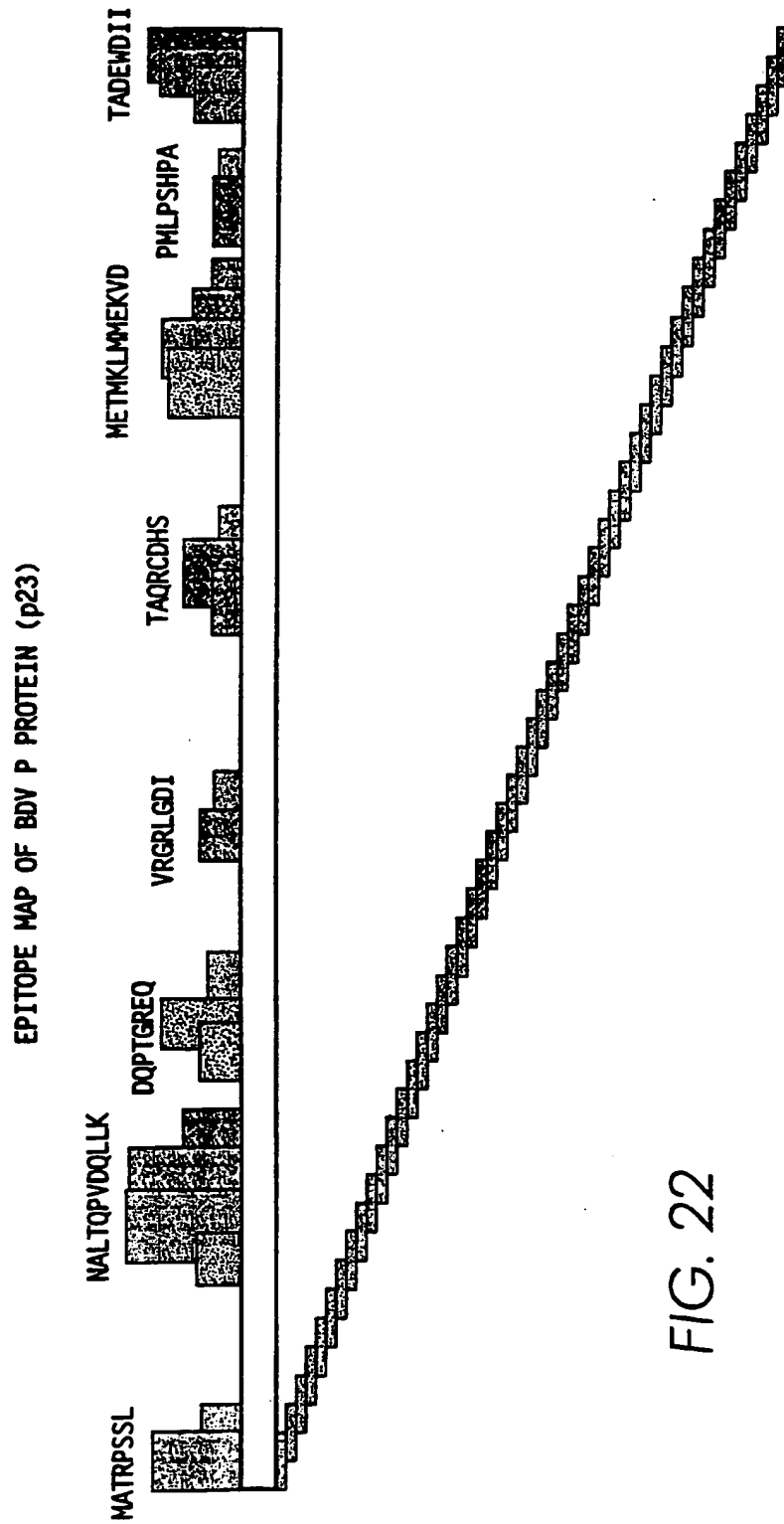


FIG. 22

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EPITOPE MAP OF BDV M PROTEIN (gp18)

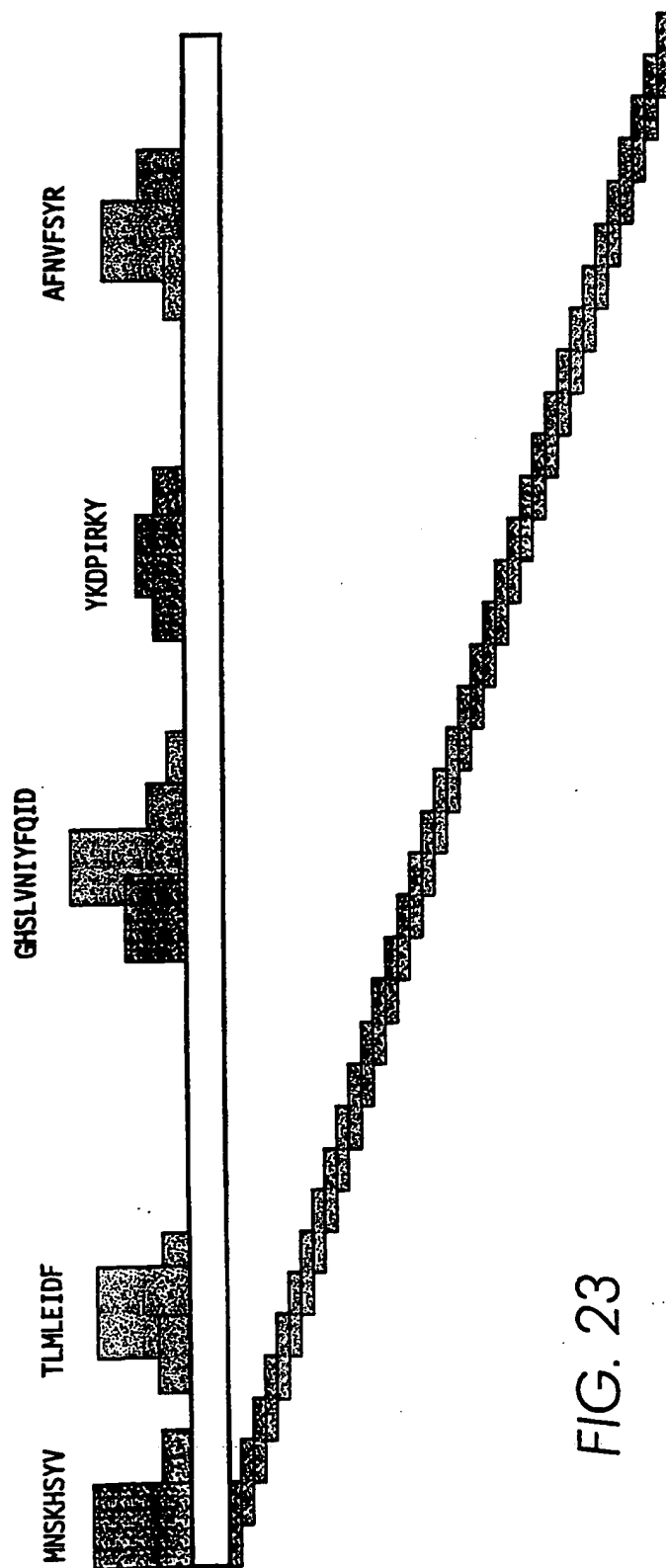


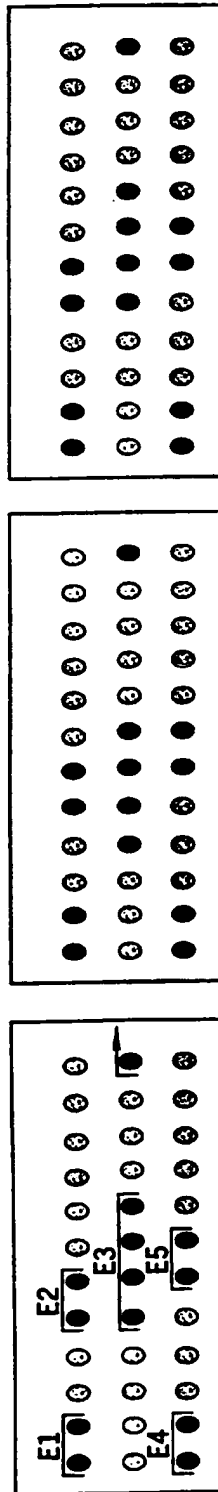
FIG. 23



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FIG. 24A

BORNA DISEASE VIRUS gp18 PEPTIDES DETECTED BY SERA  
FROM IMMUNIZED MICE, INFECTED RATS AND SCHIZOPHRENIC PATIENTS



HUMAN WITH SCHIZOPHRENIA

RAT INFECTED WITH BDV  
(15 WEEKS p.i.)

MOUSE IMMUNIZED WITH gp18

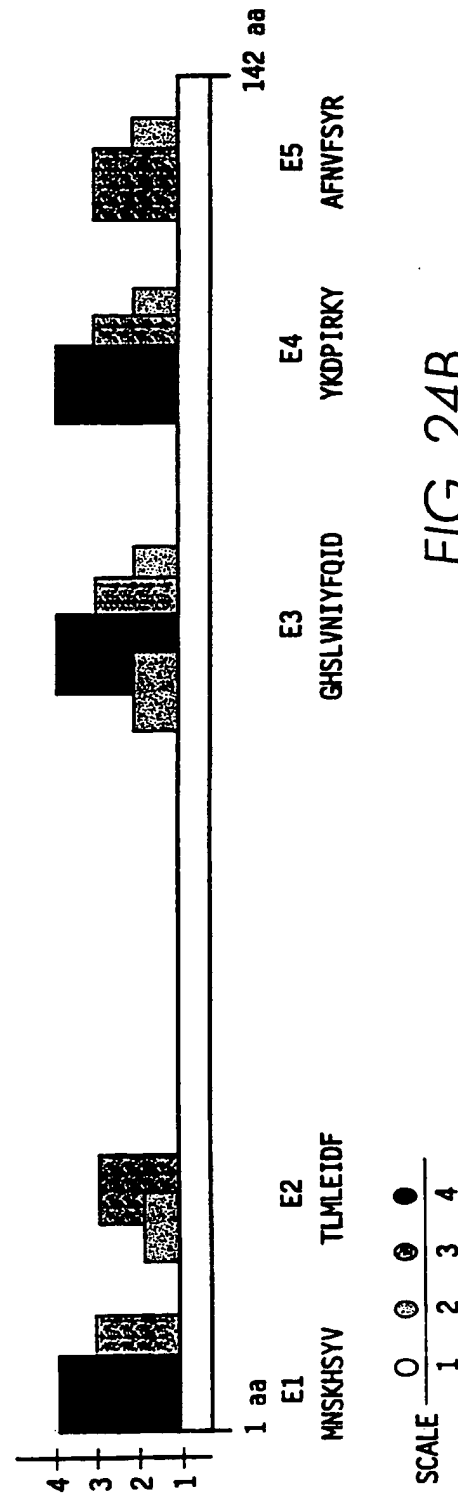


FIG. 24B

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## FIG. 25

1 MQPSMSFLIGFGLVLVLSARTFDLQGLSCNTDSTPGLIDLEIRRLCHTP  
51 TENVISCEVSYLNHTTISLPAVHTSCLKYHCKTYWGFFGSYSADRIINRY  
101 TGTVKGCLNNSAPEDPFECNWFYCCSAITTEICRCSITNVTVAVQTFPPF  
151 MYCSFADCSTVSQQELES GKAMLSDGSTLTYPYILQSEVVNKTLNGTIL  
201 CNSSSKIIVSFDEFRRSYSLTNGSYQSSSINVTCANYTSSCRPRLKRRRRD  
251 TQIEYLVHKLRPTLKDAWEDCEILQSLLLGVFGTGIASASQFLRSWLNH  
301 PDIIGYIVNGVGVVWQCHRVNVTFMAWNESTYYPPVDYNGRKYFLNDEGR  
351 LQTNTPEARPGLKRVMWFGRYFLGTVGSGVKPRRIRYNKTSHDYHLEEF  
401 ASLNMTPQTSIASGHETDPINHAYGTQADLLPYTRSSNITSTD TGSGWVH  
451 IGLPSFAFLNPLGWLRDLLAWAAWLGGVLYLISLCVSLPASFARRRRRLGR  
501 WQE

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